UNIVERSIDADE DE LISBOA

Faculdade de Farmácia

DEVELOPMENT OF A TOPICAL ANTI-INFLAMMATORY FLURBIPROFEN GEL

Dissertação elaborada para a obtenção do grau de Mestre em Engenharia Farmacêutica

Lisboa 2016

Joana Raquel Baptista de Brito Martins

Orientadores: Professora Doutora Helena Margarida Ribeiro e Doutora Sara Raposo

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Abstract

All over the years, anti-inflammatory therapy is being related to non-steroids anti-inflammatory drugs (NSAIDs). This therapeutic class has proved promising on the market once it decreases systemic adverse effects. In Portugal, there is actually 59 anti-inflammatory drugs for topical use allowed to be commercialized (*Autorização de Introdução no Mercado* – AIM) by Infarmed under the dosage form of gel. The main aim of this work was a topical non-steroid anti-inflammatory hydrogel development. There is no product with topical application, on the Portuguese market, with flurbiprofen. The patent describing Froben ® manufacturing, commercialized by Abbott, was used as a model. This product describes a formulation with 5% of flurbiprofen.

It was developed drug solubility studies, polymer quantity and cutaneous promoters selection. For that, a Quality by Design (QbD) approach was used. The menthol and oleic acid were used as permeation enhancers because they are associated to permeability and dermic retention increase.

After that, final formulations were developed, and the skin permeation and retention studied. The results were compared with the oleogel formulation described by the patent. Stability studies described on ICH Q1A (R2) guideline were also performed. Results concluded that hydrogel formulation was well succeeded. The formulation met the percentages of the patent (5% of API, 84.11% of solubilizing system, 2.5% of polymer and the remaining in permeation enhancers and solubilizing). Apart from that, the *in vitro* studies showed a higher percentage of flurbiprofen on bellow layers of the skin. A higher concentration of drug in this layers is desirable dual to their peripheral effect.

Keywords: Non-steroid anti-inflammatory drugs; topical delivery; permeation enhancers; hydrogel formulation; design of experiments; quality by design; flurbiprofen.

Resumo

A terapia anti-inflamatória tem estado, ao longo dos anos, diretamente relacionada com o uso de anti inflamatórios não esteroides (AINEs); contudo, o uso desta classe terapêutica para uso tópico tem vindo a ganhar particular destaque no mercado pois permite a redução de efeitos adversos sistémicos, como por exemplo, reações gastrointestinais. Atualmente em Portugal encontram-se registados 59 medicamentos anti inflamatórios para uso tópico com autorização de introdução no mercado (AIM) pelo Infarmed sob a forma farmacêutica de gel.

O presente trabalho visa o desenvolvimento de um hidrogel anti inflamatório não esteroide para uso tópico. Uma vez que não existe qualquer produto de aplicação tópica, no mercado português, com o princípio ativo de flurbiprofeno, esse será o objeto deste estudo, recorrendo a uma patente que descreve o processo de fabrico do produto em questão, tendo como base o Froben ® comercializado pela empresa Abbott. Este produto descreve uma formulação contendo 5% de flurbiprofeno, na qual, são utilizados promotores cutâneos de forma a promover a permeação do fármaco.

O primeiro dos objetivos deste projeto consistiu no desenvolvimento galénico da formulação tópica de flurbiprofeno no qual se englobam os estudos de solubilidade do fármaco, seleção da quantidade de polímero e seleção dos promotores cutâneos, este último, recorrendo a uma otimização por *Quality by Design (QbD)*. Após desenvolvidas e caracterizadas, foi também estudada a permeação e retenção do fármaco na pele, comparando os resultados obtidos com a referência deste trabalho; posteriormente, estudos de estabilidade recorrendo à *guideline* ICH Q1A (R2). Os resultados obtidos permitem concluir que a formulação de um hidrogel foi bem-sucedida, uma vez que, conseguiu-se desenvolver um produto que cumpria com as percentagens descritas pela patente analisada (5% de principio ativo, 80.11% de sistema de dissolução do API, 2.5% de polímero e o restante com promotores cutâneos e solubilizantes). Para além disso, os estudos *in vitro* de permeação e retenção demonstraram a maior percentagem de retenção do princípio ativo em camadas inferiores onde se pretende que haja maior concentração devido ao seu efeito periférico nas camadas inferiores; o uso de promotores cutâneos como o mentol e o ácido oleico poderão estar associados com o aumento da permeabilidade aumentando assim a retenção dérmica.

Palavras-chave: Anti-inflamatórios não esteroides; aplicação tópica; promotores cutâneos; formulação de hidrogel; *design of experiments; quality by design*, flurbiprofeno.

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Abbreviations & Symbols

% - Percentage

µg/mL – Microgram/milliliter

µm – Micrometer

AFA - Anti-filaggrin antibody

AIC – Akaike information criterion

AKA- Anti-keratin antibody (AKA)

ANOVA – Analysis of Variance

Anti – CCP - Anti-citrullinated protein antibody

APF - Anti-perinuclear factor (APF)

API – Active Pharmaceutical Ingredient

BHT – Butylated hydroxytoluene

mPa.s – millipascal seconds

CQA – Critical Quality Attributes

CPP – Critical Process Parameters

DMARs - Disease-modifying antirheumatoid drugs

DoE – Design of experiments

ED – Viable Epidermis and Dermis

EU – European Union

EULAR - European League against Rheumatism

FDA – Food and Drug Administration

g/mol – gram per mole

G-CSF - Granulocyte colony stimulating factor

GMP – Good Manufacturing Practice

h – Hours

HPC – Hydroxypropyl cellulose

HPMC – Hydroxypropylmethyl cellulose

ICH – International Conference on Harmonization

IFN - Interferon

IL - Interleukins

Kp – Permeability coefficient

Log P – Partition coefficient

mg – Milligram

mg/mL – Milligram/milliliter

mL – Milliliter

mm – Millimeter

n – Number

nm – Nanometer

NSAIDS – Nonsteroidal anti-inflammatory drugs

O/W – Oil/Water

p – P value

PEG – Polyethylene glycol

PG – Propylene glycol

QbD – Quality by Design

QTPP – Quality Target Product Profile

R^2 – Coefficient of Determination

RA – Rheumatoid Arthritis

RF - Rheumatoid factor

rpm – Rotations per minute

SB – Stratum Basal

SC – Stratum Corneum

SD – Standard deviation

TNF- α - Tumor necrosis factor alpha

v/v – volume per volume

W/O – Water/Oil

w/w – Weight by weight

WHO – World Health Organization

1st Chapter - Introduction

1. Introduction

1.1. Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a systemic autoimmune disease that begins in the interior of the synovial membrane on the joints (like hands, feet, knees and wrists). The synovial membrane surrounds the joint and handles the synovial liquid. Synovial liquid nourishes the cartilage (if healthy, must present a smooth surface) and lubricates the joints. The membrane inflammation is caused by a cellular accumulation on the site of the injury, and, there is a volume increase. This increase leads to cartilage and bones damages, causing physic deformity and a progressive physic incapability. The redness on the affected area inflammation is caused by the continuous increase of the blood flow [1] [2] [3].

1.1.1. Epidemiology

The RA cause remains unknown, however, it is estimated that the adult population up to 70 years may suffer from this condition. After this age, the trend is to decrease the incidence of the pathology [4]. According to a study, RA affects approximately 2 to 3% of the adult population and concludes that women presents more than double of the probability to develop this condition than men [5]. An epidemiologic study based on the Portuguese population, between 2011 and 2013, revealed a prevalence of rheumatologic diseases of 0.7% (n=3877) with a higher percentage for women (1.1%) than men (0.3%). This results are similar with the data provided by the European Commission [6], that presents a prevalence of 0.3% to male (n=224025) and 1.2% for females (n=68124), with a sampling of 7 countries in the European Union [7]·[8]. The World Health Organization (WHO) reports that the prevalence of this condition ranges from 0.3% to 1%, being most common on women [9].

1.1.2. Pathogenesis

Although the origin of RA is still not clear, it is known that it can due to environmental factors like smoking, epigenetic modification and genetic susceptibility. The RA begins with a synovial membrane inflammation (synovitis), and can lead to rheumatic nodules, vasculitis, pericarditis, and systemic manifestations such as anemia, cardiovascular diseases, osteoporosis, fatigue and depression [10], [11].

.

Figure 1: Rheumatoid arthritis evolution: immune system attacks the synovial membrane that results in joint inflammation and warm feeling. Certain enzymes and chemicals may be released. (Adapted from: [12])

The organism first reaction consists on the infiltration of leucocytes in the synovial membrane (Figure 1). This immunologic response it is lead by T cells production, B cells, monocytes, macrophages, and lymphocytes T. Lymphocytes T activation produces pro-inflammatory cytokines that are responsible for leucocytes infiltration and inflammation [13] [10].

An unbalanced cytokines production conducts to inflammatory processes and damages in healthy tissues. This can promote autoimmunity [14]. Although T helper cells 1 importance are clarified, it is growing the focus on T helper cells group 17, where including the cytokines IL-17, IL-21, IL-22, IL-26, granulocyte colony stimulating factor (G-CSF) and tumor necrosis factor alpha (TNF)-α [14] [11]. One of the most important group 17 pro-inflammatory cytokines it is the TNF- α. This cytokine has been revealed as an important marker in the inflammatory response. For another hand, bone and joint destruction are straight related to IL-1 [14], [15]. Table 1 describes the cytokines that may be related to RA pathogenesis:

Table 1: Involved cytokines on RA pathogenesis. (Adapted from: [14])

T helper cells	Cytokines required to maturation	Produced cytokines	Role in RA pathogenesis
Th1	IFN$_{\gamma}$, IL-12	IL-2, IL-12, IL-18, IL-7, IFN$_{\gamma}$	- Pro-inflammatory response - Activation of macrophages and neutrophils - Inhibition of Th2 lymphocytes proliferation
Th2	IL-2, IL-4, IL-31, IL-33	IL-4, IL-5, IL-6, IL-9, IL-10, IL-13	- Stimulation of B cells to proliferation and plasma cells differentiation- autoantibody production - Inhibition of Th1 lymphocyte proliferation
Th17	TGF$_{\beta}$, IL-1β, IL-6, IL-21, IL-23	IL-17, IL-21, IL-22, IL-26, GM-CSF, TNFα	- Pro-inflammatory response - Local inflammatory response regulation
Th22	TNFα, IL-6	IL-22, IL-13, TNF	- Increasing production of neutrophil attractants - Regulation of immune response

Also autoantibodies production like rheumatoid factor (RF) and anti-citrullinated protein antibody (anti-CCP) are an important tool for the diagnosis of RA [14].

1.1.3. Diagnosis

Despite all evident damages caused by RA on patients, as pain, joint deformations, and fatigue, the pathology can be medically diagnosed, by biochemical markers, or by radiology.

As mentioned before, autoantibodies produced by the organism are used as RA markers. Although RF and anti-CCP are highlighted by their strong application, the antiperinuclear factor (APF), anti-keratin antibody (AKA) and anti-filaggrin antibody (AFA), are auto antibodies that can also be used as markers. RF is an IgM antibody specific to Fc portion of IgG that is normally detected in approximately 80% of patients with rheumatoid arthritis. Anti-CCP, is produced by the arginine aminoacid conversion in cittruline, and when the test is

positive, it is very likely that the person comes to suffer RA. If the test is positive for RF and anti-CCP, the probability increases. Erythrocyte velocity sedimentation rate is also an applicable marker in this cases because some plasmatic proteins can influenced erythrocyte aggregation. The reactive C protein responsible for regulation of the inflammation intensity in the acute phase, can also be used as an applicable marker.

The European League against Rheumatism (EULAR) recommends the use of imagiologic techniques against RA such as conventional radiographic, ultra sounds, magnetic resonance image, X-rays, scintigraphy and positron emission to tomography [16] [17].

1.1.4. Treatment

After diagnosis, patient must start a proper treatment according his condition. Although RA cure remains unknown, there are a set of treatments that aims to decrease pain and inflammation [18]. From all the treatment options, the most common it is the drug treatment with some therapeutic classes available. Other therapies are available such as physiotherapy, podology, occupational therapy and hydrotherapy. In some situations, cirurgic procedure can be an alternative [19].

1.1.4.1. Disease-modifying anti-rheumatoid drugs (DMARDs)

DMARDs can be biologic or non-biologic agents able to reduce symptoms and reverse the damage progress in the joints [20]. DMARDs helps to preserve the joints acting on the inflammation blocking. DMARDs are usually prescribed in the early stage of RA combined with NSAIDs for inflammation blocking and pain relief. In the biologic class of DMARDs, it is included the monoclonal antibodies and receptors responsible for the blocking of some chemical mediators such as cytokines already referred in the inflammation process (for example, the TNF- α). The non-biologic DMARDs are known as low molecular weight and comprises a large range of chemical drugs (such as methotrexate, the quickest acting DMARDs used) [21].

The use of this DMARDs improves the global functional capacity while reducing radiologic damages, clinic manifestations of the inflammation and slowing of the disease progression. However, this class can be related to some adverse effects. Biologic DMARDs, presents some immunologic adverse effects due to infection resistance by the immunologic system inhibition, when the monoclonal antibodies blocks the immunity response allowing the appearance of opportunity infections. The non-biologic can take three to six months to be effective and most of them are tablets, leading to gastrointestinal adverse effects.[19] [22] [18].

1.1.4.2. Corticosteroids

Corticosteroids are small hydrophobic molecules with anti-inflammatory function responsible for blocking inflammatory substances such as prostaglandins and leukotrienes [16]. This drugs used for symptoms decrease are administered by injections (intravenous or muscular route) or tablets in a short period due to the adverse effects of the drugs. Steroids injections can cause changes in humor, menstrual cycle and also changes in the site of injection administration while tablets can induce weight gain, muscular fading, cataracts, blood flow increased and infections vulnerability [17].

1.1.4.3. Non steroid anti-inflammatory drug (NSAIDs)

This therapeutic class of non-steroid anti-inflammatory drugs is responsible for the cycle oxygenase (COX) inhibition, an enzyme that is involved in prostaglandins production. It exists in two forms: COX 1 and COX 2. COX 1 is normally expressed under basal conditions and it is related to prostaglandins synthesis; the COX 2 is higher during the inflammatory process and others pathologic situations. The selective inhibition class of COX 2 is related with prostaglandins inhibition in first place, while the remaining class is responsible for the prostaglandins synthesis inhibition in other places of human bodies, such as stomach, where they performs a protective role. However, the use of this class can lead to another problem like cardiovascular diseases because their utilization has an impact on the balance of prostacyclin PGI_2 and thromboxane A_2, essential to cardiovascular health. The NSAIDs can be grouped according to chemical structure, biologic half-time and the selective of COX 1 vs COX 2. In table 2 there is all the classes and the most common NSAIDs [16], [23] [24]:

Table 2: Non steroid anti-inflammatory drugs classes. (Adapted from: [23] [25])

Class	Subclass	Drug
Carboxylic acids	Salicylic Acids	Acetylsalicylic acid
		Diflunisal
		Trisalicyliate
		Salsalate
	Acetic acids	Diclofenac
		Etodolac
		Indomethacin
		Sulindac
		Tometin
		Ketorolac
	Propionic acids	Flurbiprofen
		Ketoprofen
		Oxaprozin
		Ibuprofen
		Fenbufen
		Zomepirac
		Indoprofen
		Naproxen
		Phenoprofen
	Fenamic acids	Meclofenamate
Enolic acids	Pyrazolones	Phenilbutazone
	Oxicams	Piroxicam
		Meloxicam
Nonacidic		Nabumetone
COX-2 selective	Sulfonamide	Nimesulide
		Celecoxib
	Sulfonylurea	Etoricoxib
	Nonacid	Lumaricoxib

NSAIDs are usually used as analgesics for the inflammation symptomatic reduction while patient does not show damages caused by RA like bone damages, mobility loss, joint swelling, and others. Although NSAIDs are a low toxic therapeutic, it can also express some toxicity in the following organs (Table 3) [16], [23] [26]:

Table 3: Toxicity caused by NSAIDs use. (Adapted from: [23])

Organs system	Toxicity
Gastrointestinal	Dyspepsia, esophagitis, gastroduodenal ulcers, ulcer complications (bleeding, perforation obstruction), small bowel erosions and strictures, colitis.
Renal	Sodium retention, weight gain and edema, hypertension, type IV renal tubular acidosis and hyperkalemia, acute renal failure, papillary necrosis, acute intestinal nephritis, accelerated chronic kidney disease.
Cardiovascular	Hearth failure, myocardial infarction, stroke, cardiovascular death.
Hepatic	Elevated transaminases
Asthma/alergic	Aspirin-exacerbated respiratory disease, rash-
Bone	Delayed healing

Despite NSAIDs toxicity, this therapeutic class is very used in transdermal and topical formulations development due to analgesic anti-inflammatory action associated to pain relief. Furthermore, most of NSAIDs have ideal physical and chemical characteristics for topical application: low water solubility (< 1 mg/mL), lipophilicity (log P=1-3), low molecular weight (< 500 Dalton) and low melting temperature (< 200 ºC). Topical NSAIDs formulations can lead to pain relief on local inflammation and adverse effects reduction of oral formulations [25].

1.1.4.3.1. NSAIDs in national market

According to *Infarmed* website, 89 products are registered at *Infomed* [27] as topical gels (that will be the target in this study) belonging to non-steroid anti-inflammatory class for topical use (section 9.1.10 of therapeutic class). From this only 59 are allowed to be commercialized, the 30 remaining are with rejected or expired status. From this list, there is also presented another NSAIDs, being the most common, the Diclofenac.

1.2. Dermal application systems

Dermal application pharmaceutical development had been focused in the pharmaceutical conventional class such as gels, creams, lotions, emulsions and transdermal patches. However, new pharmaceutical formulations for transdermal release and delivery has been increasing. Beyond this gap between old and new formulations, a new division between the used methodologies emerged: active or passive. The active method allows molecules transport with molecular weight higher than 500 Da, which means transdermal distribution significantly advances. Also in active methods (or physic methods) are included techniques like ultrasounds, electrically assisted methods (like elephoration and iontophoresis) and laser. This method presupposes the external energy use as a drug transport force through the skin. At least, in passive methods cases (or chemical) they are influenced by the active method, transport vehicle and the interactions between them, leading to formulation optimization [28].

The main vantage of topical formulations it is the secondary effects reduction. The first pass effect is also avoided. A high compliance and prolonged release during a long period in the action local makes this therapeutic a very used one [29] [25].

1.2.1. Dermal barrier

The skin allows an action against potential noxious agents but prevents the exit from the endogenous material of the organism. His main function is protection, but is also responsible for body homeostasis, regulation of corporal temperature and blood flow pressure. This barrier is categorized in 3 dermic levels such as epidermis, dermis and hypodermis (Figure 2):

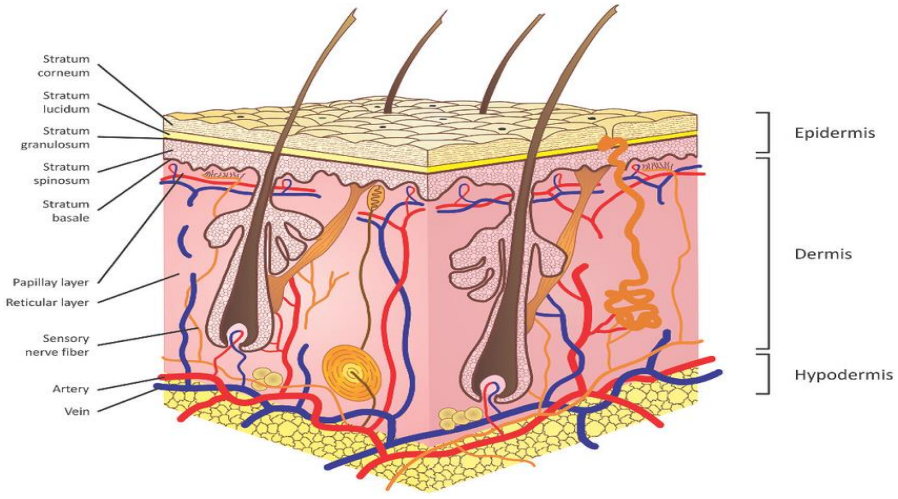


Figure 2: Dermal barrier representation (Retired from: [30])

Epidermis is the most external layer and its constitution are mostly keratinocytes (95%). They will suffer differentiation and maturation, ascending to corneocytes, in a migration process since *stratum basal* (SB) until the most out layer named *stratum corneum* (SC). The remaining epidermis is composed of Langerhans cells, melanocytes and Merkel cells. Excluding the SC, the remaining epidermis is called by the viable epidermis and is devoid of blood capillaries. Beyond the SC and SB, there is also 3 other layers that forms epidermis: *stratum lucidium, stratum granulosum* and *stratum spinosum*. This mechanism of keratinocytes cell proliferation until the SC lasts about 28 days and allows the cellular renovation, after that, the cells suffers dehydration, flatness, and their nucleus disappear, increasing the keratin content in the SC cells that will be immersed in a lipid emulsion with a pH from 5 to 5.5 [31], [32]

This layer is very important once would be necessary a formulation according to her characteristics. Only relatively lipophilic compounds will pass through SC for the most interior skin layers [29] [25] [33]. However, drug absorption for the systemic current depends on SC drug passage to interior layers such as dermis, that is essentially aqueous which means a new formulation development with hydrophobic and hydrophilic characteristics [34].

Dermis represents the bigger portion of human skin and it is primarily constituted by fibroblasts, macrophages and mastocytes. Fibroblasts are responsible for collagen synthesis and the other two for immune functions. This layer is very vascularized and it is responsible for human temperature regulation [31], [32].

The hypodermis is the most interior layer and it is mostly constituted by fatty cells surrounded by conjunctive tissue, being essential in protection against physical injuries [31].

1.2.1.1. The barrier

The “brick and mortar” model is often used to explain the *straum corneum* layer where corneocytes are compared to bricks and intercellular lipids the mortar. This membrane continues to be the major problem of drug permeation once that it is mostly composed by corneocytes surrounded by a complex of lipids arranged in bilayers that may difficult the passage of drug molecules. This hypothesis is supported by some literature that referred the elimination of lipids, or the disruption with appropriate permeation enhancers, for improve SC drug permeation. The drug permeation can through SC membrane by 2 different pathways: transepidermal (including intracellular and/or extracellular) and appendageal. (Figure 3):

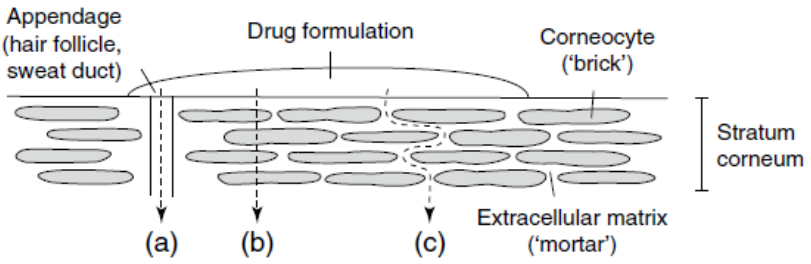


Figure 3: Drug permeation pathways in the skin: a) appendageal route, b) transcellular route and c) extracellular route (Retired from: [34])

The transepidermal pathway allows drug permeation through intracellular and/or extracellular spaces from the outsider layer into hypodermis. This pathway involves drug partitioning and diffusion on the different hydrophilic and lipophilic domains. The appendageal pathway allows permeation through hair follicles sweat duct [35] [36] [37].

Although dermal barrier complexity it is possible to describe the membrane transport through mathematical models.

1.2.1.1.1. Mathematical models for drug release kinetics

The drug permeation through the skin, presupposes the release profile study of each drug, either for permeated drug quantification or to understand the release profile through the time. Release mechanism transport can be done by diffusion, erosion and degradation. Diffusion is the most release system used in mathematical models. To describe the most properly drug release profile, some important mathematical models such as zero order kinetic model, first order kinetic model and Higuchi model are used.

Molecular diffusion it is the molecular transport through the skin surface, most precisely, passive diffusion. The flux (expressed in J – µg cm^{-2} s^{-1}) from which the drug pass through the skin is defined as the permeated amount of drug through the time and can express by the following expression:

$$J = \frac{dQ}{dt} = \frac{DPCv}{h}$$

Equation 1: First Law of Fick Diffusion.

Where Q represents the permeated amount for each area unit, D diffusion coefficient, P the partition coefficient, C_v the applied amount of permeant and h the membrane thickness. It can be measured the cumulative Q by the following expression:

$$Qn = (Cn * V0 + \sum_{i=1}^{n-1} Ci * Vi)/A$$

Equation 2: Drug cumulative amount permeated through the skin.

Where C_n represents the drug amount in the receptor media, C_i the sample concentration, A the diffusion area and V_0 and V_i the volumes on the receptor and in the sample, respectively. In the permeation of *in vitro* studies, for the success of drug passage, it is very important the choice of the receptor media, in order that he can maintain the sink conditions. Sink conditions expresses that he dissolved drug concentration in the receptor media must be 10% inferior to the saturation concentration [33] [36] [38].

From Fick's law, some parameters such as permeability coefficient (Kp) and lag time (represented in time) can represent the absorption of a compound in the vehicle and the delayed time before the compounds start to increase permeation, respectively:

$$Kp = J/Q$$

Equation 3: Permeability coefficient equation.

$$Lag\ time = h^2/6D$$

Equation 4: Lag time equation.

where h is path length for diffusion and D the drug diffusivity [36].

The Zero-order release predicts a constant drug release through time that do not degrade and had a slow release, independently of initial drug concentration (Equation 5) [39]:

$$C(t) = C0 + k0 * t$$

Equation 5: Zero-Order release model equation

Where C(*t*) it is the drug concentration released in the time *t* (mg/mL), C_0 it is the initial drug concentration (mg/mL), k_0 the velocity constant of the zero-order reactions and *t* the time in hours.[32] [40] This model describes a release proportional to the amount of drug remaining in the donor chamber.

1st order release describes a release proportional to drug concentration in the matrix [39]. This model is used to describe drug absorption and elimination.

$$lnC(t) = lnC0 + k * t$$

Equation 6: 1st order release model equation.

The Higuchi model (1961) is based on the First Fick's Law and predicts a homogeneously dispersion of the drug in matrix. Higuchi model suggests a diffusion release mechanism with no erosion or swelling of the matrix. In a general way, this model can be resumed to the following expression [40], [41] [39]:

$$Q = Kh * \sqrt{t}$$

Equation 7: Higuchi model.

Kh it is the release constant of Higuchi that reflects the formulations characteristics. The release is proportional to square root of time [42].

Variation coefficient R^2 is often used as a determinant factor to choose the most properly model. Sometimes Akaike Information Criterion (AIC) is also used as a discriminatory criterion [43].

1.2.2. Pharmaceutical development of topical formulations

To overcome the skin barrier there are two different ways: transdermal and topical. Transdermal dosage forms carries the drug through skin by permeation until drug arrives at blood circulation (transdermal patches). In topical formulations the drug will act in a specific anatomic region and the effect of this dosage form is local. Even so, both ways avoid the toxic effects already mentioned. In order to achieve their goals, some common characteristics should be taken in consideration [25] [44]. There are some common characteristics that both formulations must obey (Table 4) [45]:

Table 4: Dermal formulations characteristics.

Developed for	- Stability - Skin penetration (drug lower solubility on vehicle and maximum on skin) - Facility of passage through skin - Prolonged release - Easy repeated application - Safety, compliance
Composed by	- Anti-inflammatory steroids - Anti-inflammatory non steroids - Pain killers/anesthetics
Complements	- Preservatives - Skin permeation enhancers - Vehicle reservoir

Before pharmaceutical topical development, it is necessary to determine what the pretended product, either to be a liquid, solid or semisolid form. There is a lot of available options and these need to be considered in the moment of product development (Table 5). Beyond active principal choice, it is equally important the excipients choice. The total amount reaches to a total 90% of the final formulation. Essentially, they allows API solubilization, permeating the skin and reaching the target. For example, for aqueous preparations (an example of hydrogels and emulsions O/W) polyols are used solvents such as polyethylene glycol (PEG) and propylene glycol (PG) for solubility increase, having a fundamental role in the formulation development. The use of cutaneous permeation enhancers causes an impermeability reduction, being that in this class belongs compounds like azones, pyrrolidones, fatty acids, glycols and phospholipids. It pretends that this compounds do not have any kind of toxicity in the organism and promoting the drug absorption for local or systemic effects. Other fundamental excipients, for aqueous formulations essentially, are the antioxidants, once the final product could be oxidative degradation sensitive. However, not only oxidation can affect drug stability but also pH, once that some excipients can be affected for pH and thereafter affect, for example, viscosity [19], [22].

Table 5: Topical pharmaceutical forms (Adapted from: [33])

Topical formulation		
Liquid	**Semisolid**	**Solid**
Emulsion (O/W, W/O)	Colloid	Patches
Suspension	Cream	Powders
Spray (Propellant, pump)	Ointment	
Solution	Paste	
	Foam	
	Gel (aqueous, non-aqueous)	

Although there are many possibilities formulate topical development, the development is always changing. This study, will focus in the development of a hydrogel containing flurbiprofen.

Gels are semisolids preparations intended to the cutaneous application. In those, API are solubilized and gelling agents are incorporated, responsible for semisolid characteristics. According to the used excipients conjunct, gels can have lipophilic or hydrophilic characteristics. The 8th Portuguese Pharmacopeia defines lipophilic or oleogels like preparations where it is generally constituted by liquid paraffin added polyetilenic compounds or jellified fatty acids by colloidal silica or aluminum or zinc soaps. Hydrophilic gels, or hydrogels, are preparations where excipients are usually water, glycerin and propylene glycol (jellified with properly jellified agents, such as starch, cellulose derivatives, carbomers or magnesium-aluminum silicates). In both cases, it is important the use of antimicrobial agents, antioxidants, stabilizers, emulsifiers, thickeners, and absorption permeation enhancers [46] [36] [47] [33].

Jellifying agents have a big importance since they are responsible for gel viscosity, depending on the added quantity in the product, being usual values in the 1-5% (w/w) range. It can be used natural polymers such as casein, gum arabic and silica, synthetic polymers like methylcellulose, hydroxypropylcelullose (HPC) and hydroxypropylmetylcellulose (HPMC) and synthetic polymers like the carbomer class (ex: Carbopol®). The selected type of polymer can influenced the rheological behavior of gel, leading to physic changes, conditioning the spreading of gel in skin by the user, and a different compliance [46] [36] [47] [33] [48].

With that been said, there are some rules that gels must obey:

- ✓ Polymer must be inert, secure and not reacting with remaining excipients
- ✓ Polymers must induce a stable formulation, even exposed to external forces (ex: agitation)
- ✓ It must contain antimicrobial agents
- ✓ Do not must be sticky
- ✓ In the ophthalmologic gels, it must be sterile [49]

1.2.2.1. Topical formulations for flurbiprofen delivery

Some studies had focused on flurbiprofen non steroid anti-inflammatory drug on topical formulations. The high log P and low molecular weight make it a worthy candidate for topical delivery due good permeability among the NSAIDs usually used (Table 6) [50]:

Table 6: Log P, water solubility, molecular weight and biologic half time values for some of the most common NSAIDs. (Retired from: [51])

API	Log P	Water solubility (mg/ml)	Molecular weight (g/mol)	Biologic half time (h)
Piroxicam	3.06	23	331.3	30-86
Benzidamine	3.99 (2)	-	309.4	13 (3)
Ketoprofen	3.12	51	254.3	2-2.5 (3)
Diclofenac	4.51	2.37	296.2	2 (1)
Etofenamate	3.53 (1)	0.00936 (1)	369.3	3.3 (4)
Ibuprofen	3.97	21	206.3	2-4
Flurbiprofen	4.16	0.008	244.2	4.7-5.7
Naproxen	3.18	15.9	230.3	10-20
Nimesuline	2.6	0.0182 (1)	308.3	1.8-4.7

(1) Retired from [52]
(2) Retired from [53]
(3) Retired from [54]
(4) Retired from [55]

Partition coefficient (log P) allows a better analysis of the molecule affinity; the higher this coefficient is, the higher it is lipophilicity and the permeation through the skin. Furthermore, it is pretended to have a molecular weight the lowest possible that the small molecular weight molecules have more difficult to permeate the dermic barrier. The same thing happens with biologic half-life ($t_{1/2}$), that it is the necessary time to half elimination of the drug in the organism, and pretends to be minimum, for the drug action occurs as soon as possible. Lastly, the water solubility reveals a big importance for the solvent system choice for the pharmaceutical development.

The studies performed in the attempt of topical application products such as gels, creams and emulsions containing flurbiprofen showed low permeation due to low water solubility and permeability. However, the use of cutaneous permeation enhancers can be an alternative for increase the permeation flux of flurbiprofen through the skin [56]–[58]. Some permeation enhancers like polyethylene glycol, propylene glycol [59] [50] were used in formulation studies in order to increase the permeation of flurbiprofen. Urea, oleic acid, and isopropyl myristate were also used in studies where an increase on skin permeation was observed in all of them, encouraging the use of flurbiprofen in future formulations [58] [60].

The Froben® 5% flurbiprofen gel is the only flurbiprofen topical formulation available at the moment on the market. It is currently manufactured by Abbott company in Pakistan and is sold in countries like Egypt and India [61] [62].

1.3. Pre-formulation studies

1.3.1. API selection

The drug conception idea requires some initial steps before proceeding to the pharmaceutical development. In topical formulations, it is fundamental to have into account drug distribution since it is crucial to product success or failure. API and excipients choice must be the first step, and can be guided by the following diagram [33] [63]:

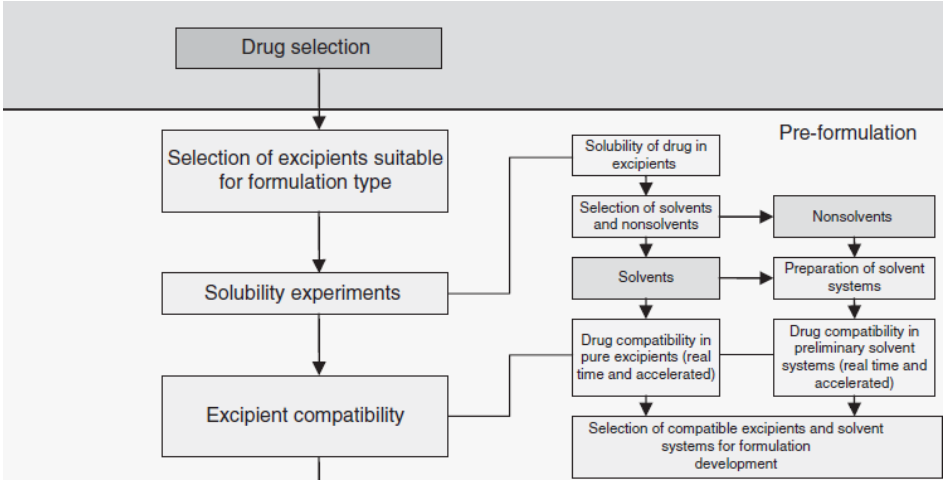


Figure 4: Diagram for adequate solvents and excipients selection for typical topical semisolid formulations development (Retired from [33]).

During pre-formulation studies, solvents choice is a very critical parameter. However, co-solvents selection is equally a critical point since they are an important strategy to improve the solubility of low soluble water compounds. Furthermore, pH is also an important to increase

solubility, considering most of API compounds are lower ionizable acids/bases, it is important to consider the pH of the solution and the pKa of the API. It is necessary, to control the pH of the final formulation, which should be situated between 5 and 7, however values starting from 4 are usually accepted [33].

The physical and chemical optimum proprieties of API must be considered, as well as its biological and pharmacodynamics/pharmacokinetic properties. So, the ideal characteristics are usually characterized by the following aspects (Table 7) [50]:

Table 7: Optimum properties for API choice (Adapted from: [63])

Parameter	Properties
Dose	Must be low (< 2mg/day)
Biological half-life (h)	10 or less
Molecular weight (g/mol)	< 400
Log P	1-4
Skin permeability coefficient	< 0.5 x 10^{-3} cm/h
Skin reaction	Not irritating
Oral bioavailability	Low

1.3.1.1. Flurbiprofen

Flurbiprofen it is a non-steroid anti-inflammatory belonging to the propanoic acid class with analgesic effect, anti-inflammatory and antipyretic effect, being used in several pathologies treatments such as rheumatoid arthritis, osteoarthritis and back pain. Chemically, it is a propanoic (RS)-2-(2-fluorobifenil-4-il) acid being his chemical form $C_{15}H_{13}FO_2$ with a molecular weight of 244,3 g/mol. Flurbiprofen it is also available in the form of salt: sodium flurbiprofen with a molecular weight of 302,27 g/mol. Flurbiprofen Sodium salt it is usually used as ocular solutions due to its higher solubility comparatively to flurbiprofen [64] [65].

Flurbiprofen appears as a crystalline powder, white or almost white, practically water insoluble, being easily soluble in alcohol and methylene chloride. Sodium fluriprofen is sparingly soluble in water, soluble in alcohol and dichloromethane. (Figure 5) [66], [67] [68]:

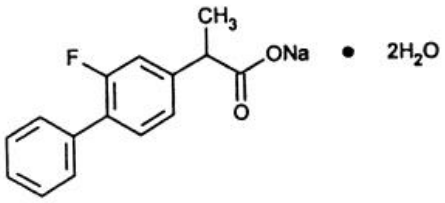


Figure 5: Chemically structure of Sodium flurbiprofen (Adapted from: [69])

Flurbiprofen possesses a chiral center, pursuant to, have 2 enantiomers: R and S. Although mostly of NSAIDs are commercialized in a racemic mixture form (i.e. with an equally proportion of both enantiomers), such succeeds with flurbiprofen being the most of the anti-inflammatory activity associated with enantiomer S [70].

Relatively to pharmacokinetic parameters, flurbiprofen presents a pKa of 4.42 (strong acid), a logP of 4.16 and a biological half-life of 4.7 hours for R-flurbiprofen and 5.7 hours for S-flurbiprofen, respectively. Presenting a biological half-life relatively short comparing to the other NSAIDs and a higher logP, which traduces in a highly lipophilic compound, make them strong candidate to topical development [71].

The oral flurbiprofen administration is responsible not only for several secondary effects such as abdominal discomfort, gastrointestinal effects (intolerance and ulcers) and constipations, but also for the first passage effect, very common in oral drugs.

1.3.2. Excipients selection

Excipients choice in topical formulations is a very critical moment not only due to their capacity to influence drug stability, but also due their capacity to modulate skin barrier function. The selection of excipients for the formulation can be based on literature, patents or existing formulations where most of the concentrations are optimized. The use of raw materials will depend from pharmacopeias and that must be considered. The use of water as a solvent is very common in aqueous gels and O/W emulsions, due to cost effectiveness, and, facility to mix with other water-miscible solvents to increase drug solubility (such as PEG, PG, alcohol, etc), to allowing the desired effect on the target concentration. Also the use of fatty acids, alcohols, glycols and surfactants can play an important role as co-solubilizers in aqueous formulations. The duality of the most of the excipients is very common, having multifunctional properties in the formulation. For example, some humectants such as polyols (PG, PEG) can also be incorporated in aqueous formulations such as gels to improve the moisturizing effect, comparing to creams and ointments, being at the same time solvents.

The SC barrier function can be a problem resulting in a reduction of permeation of topical drugs. SC it is a very selective layer and therefore, does not allow the permeation of all molecules, being selective to molecules with specific physicochemical properties. This drawback can be overpassed by permeation enhancers use. Some permeation enhancers classes like azones (Azone®), pyrrolidones, fatty acids (oleic acid), alcohols (ethanol), glycols, surfactants, phospholipids, esters, amines and amides (urea, dimethylacetamide, dimethylformamide), terpenes, etc, can be used to disrupt the skin barrier properties,

increasing drug partitioning into the various skin layers and to modifying intracellular protein domains. Although there is other permeation enhancers, the use of chemical permeation enhancers represents the most studied method and as demonstrated, covering a wide drug range since lipophilic to hydrophilic drugs. [35] [33] [72] [73] [74].

1.4. Formulation Development

During pre-formulation studies, the main concern is to focus on adequate excipients with acceptable concentrations and their interactions with the selected drug. In another hands, the formulation studies may lead to a formulation selection based on previous existent pre-formulation studies, the stability of the formulation and selection of the formulations to skin permeation and release studies. Stability studies provide a knowledge about the influence of some factors such as temperature, humidity, and light on drug product, and, are performed according to ICH Q1 (R2) guideline – Stability Testing of New Drug Substances and Products -, which define the storage conditions according to climatic zones:

Table 8: General case for storage conditions of new products (Adapted from: [75])

Study	Storage condition	Minimum time period covered by data at submission
Long term	25ºC ± 2ºC/60% RH ± 5% RH or 30ºC ± 2ºC/65% RH ± 5% RH	12 months
Intermediate	30ºC ± 2ºC/65% RH ± 5% RH	6 months
Accelerated	40ºC ± 2ºC/75% RH ± 5% RH	6 months

*RH – Relative humidity

The container where the drug product would be packaged should be the same as proposed from marketing, and, the data collected from stability studies should provide at least three batches from the product. The batches should be replicated from the same formulation. Furthermore, in an accelerated study, a minimum of three time points is recommended (including the 0 and 6 months). An accelerated stability study provides information about the degradation drug substance and possible chemical and physical factors that affect degradation. This type of information are very useful to formulation factors that may affect product stability. At each data time analysis, a role of tests should be performed such as pH, physical appearance (macroscopic) and drug content [75] [33] [76] [74].

In vitro release and permeation studies are largely used in order to study skin penetration of the drug product. The *in vitro* techniques are mentioned as better techniques than *in vivo.* In the first one*,* the drug quantity it is directly measured under the skin surface, while *in vivo* quantifies the systemic concentration of the permeant. However, *in vitro* studies can not perform the variability of the skin and it is only conditioned by two variables: skin and test material. Usually, static diffusion cells (Franz cells), are used to study absorption from semisolid preparations composed of two compartments: a donor and a receptor compartment. A permeant and a receptor fluid (pH 7.4 phosphate-buffered saline) are applied. The skin or membrane should be applied between the two compartments and the assay must be at 32 ± 1ºC for a minimum of 24 hours [77] [74].

1.4.1. Product Design

In the earlier stages of a formulation development a quality target product profile (QTPP) is normally used to define the pretended product. QTPP expresses the desired product characteristics in clinical, pharmaceutical, technical, regulatory and commercial/marketing terms and should be adequate to all customer and end-user needs. The pharmaceutical development pretends to design a product according to recommendations and norms, to ensure the maximum possible quality; for that, the ICH Q8 (R2) says that all experience gained through pharmaceutical development studies is fundamental to gain experience and knowledge to create a Design Space. The Design Space (DS) allows a combination of the input variables and the process parameters, establishing mathematical relationships between them. The elements present in a documentation submission to new pharmaceutical product development can be varied according to the company, however, there is some recommended elements by this guideline:

- Quality Target Product Profile: list with product quality, safety, and efficacy taking into account the administration route, dosage form, bioavailability and stability;
- Critical Quality Attributes (CQAs): product characteristics that reveal impact in product quality, to be studied and controlled;
- API and excipient CQAs determination and selection of the type and quantity of the desired quantity of the product;
- Selection of an adequate production process;
- Targeting a control strategy [78].

1.4.2. Product Optimization

A product optimization ensures that product complies with the stipulated characteristics on design stage. The ICH Q8 guideline has as objective an experimental design studies development about an in-development product and here process production, from which can be represented in a DS. The DS results on a combination of inputs variables (such as, material attributes), and process parameters and define the optimum operation zones.

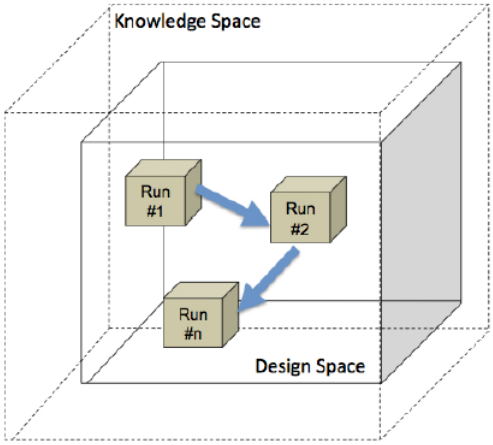


Figure 6: Concept of Design Space (Adapted from: [79])

The experimental data analysis can be taken into account to establish an ideal operation area, where the target definitions result in a high-quality product production. This idea of DS is based on Experimental Design, an experimental methodology introduced to allowing a variation of more than one single factor at a time-saving time, money, drug substance, identifying interaction effects and characterizing a response surface. A response surface allows varying process variables and understand how process responds to that variations. [74] The use of experimental data can be very helpful to optimize a product during his process step and, for that, there is an increasing focus on design techniques use, who can achieve a rapid and successful development of the product. The design techniques are often used to identify critical process parameters (CPPs) who could lead to the failure or success of the product and define the acceptable range to operate in it. Some approaches such as Design of Experiment (DoE), process analytical technology (PAT) and risk assessment can be used to identify potential CPPs [80].

Overall process product there is a big range of factors that can influence the quality of the product. It is almost impossible to study all them and their input and output variables. DoE methodology can be a powerful tool in that way, since that combines prior knowledge and risk

management, and it is possible to combine key variables leading to the identification of critical factors associated with CQAs [80].

Quality risk management is introduced by ICH Q9 guideline – Quality Risk Management – and focus on identifying risks and analyzing them. Risk analysis notion introduction is connected with GMPs due to quality risks, safety, and efficacy. The use of risk assessment during all product development can be very useful combined with previous knowledge and experimental design data to identify critical and noncritical parameters and attributes. According to ICH Q9, Quality Risk Management is described as a powerful component of quality system. This principle is applied not only to the final product, as also to during all production process. This guideline following two principal principles:

- Quality risks must be based on a scientific knowledge and interconnected to the patient protection;

- The effort level, formality, and risk management process documentation must be proportional to the risk level.

A risk management model usage is recommended through a diagram, in what should be considered all process elements, with respectively associated risk. Some of the techniques recommended by ICH Q9 for risk analysis are flow charts, check sheets, process mapping and cause-effect diagrams (Ishikawa diagram) [81]–[83] [74].

The final formulation optimization can include some approaches such as factorial design, single-factor and systemic; the factorial design is often used in product development when some excipients can interact by themselves with significance, simultaneously. This approach is generally limited to a few experiments and a limited number of variables. A single-factor approach involves a variation of one single parameter and it is generally used to experienced formulators [33] [36] [84].

Quality control in pharmaceutical industry is one of the most important, and is not surprising the increasing emergence of restrictions to pharmaceutical development. The concept of Good Manufacturing Practices (GMP) comes to assure not only the final product quality but also the quality through all life cycle, obeying to regulations and guidelines. Although the existence of diverse regulatory entities depending on the country, the guidelines of Food and Drug Administration (FDA), the International Conference of Harmonization (ICH), World Health Organization (WHO) and European Union (EU) are cited as the most influence and frequently referred in literature [81], [85].

2nd Chapter – Pre-formulation studies

2. Introduction

In this chapter, a pre-formulation approach was developed. The strategy was intended to study an innovator hydrogel formula by having a reference an oleogel formulation. Several solvents, as well as the remaining excipients (polymer concentration, permeation enhancers, and solubilizers), were studied, with the purpose of developing a final stable formulation. A QbD approach was developed in order to support the optimization of the formulation including some tools such as DoE and a QTPP.

2.1. Materials

Sodium flurbiprofen was a courtesy from Laboratório Edol Produtos Farmacêuticos SA (Linda-a-Velha, Portugal) as isopropyl myristate, propylene glycol, phosphate buffer pH 7.4 and lactic acid 10%. The etoxydiglycol (Transcutol® P), dyperlagonate propylene glycol (DPPG® CG) and the propylene glycol 8 caprylic (Labrasol®) were gently given by Gattefossé (France). The ethanol absolute anhydrous was supply by Carlos Erba (Val de Reuil, France), Klucel® from Hercules (Wilmington, USA), oleic acid from Merck (Germany), Tagat® CH 40 (PEG-40 Hydrogenated castor oil) from Evonik Industries AG (Essen, Germany) and menthol crystals were provided by DS Produtos Químicos (São Domingos de Rana, Portugal). Purified water was obtained by reverse osmosis, electrodeionization (Millipore, Elix® 3) followed by filtration (0.22 µm). SIL-TEC® (silicone membrane) was purchased from Technical Products (Georgia) and PVC tubing 0.8 x 2.4 mm from Kartell (Italy).

2.2. Equipments

The below equipments were used in the pre-formulation studies:

- Stir Plate, *RH basic 2® IKA (Staufen, Germany); VMS-A® VWR (USA)*
- Analytical Balance, *AG204® Mettler Toledo; KN 3600-2N Kern® (Balingen, Germany); AE 260® Mettler Toledo (Switzerland)*
- Ultrasonic Bath, *Branson 8210® Bransonic (USA)*
- Viscometer, *Brookfield® DV II + Pro, Brookfield Engineering (USA)*
- Centrifuge, *Z 400K®, HERMLE (Wehingen, Germany)*
- Potentiometer*, 827 pH lab® Metrohm (Switzerland); Seveneasy® Mettler Toledo (Switzerland); inolab pH 730® VWR (USA)*

- Chromatographic system*, Hitachi Elite®, VWR (USA)*

2.3. Methodology

High Performance Liquid Chromatography

Flurbiprofen assay

1.1) Standard solutions preparation

For the standard solutions preparation 22.5 mg of sodium flurbiprofen was weighed into a 50 mL volumetric flask. Methanol:water (50:25, v/v) was added at approximately 1/3 of the total volume. The solution was placed in the ultrasounds bath for 5 minutes. The final volume was completed with the solvent. From this solution, 2 mL was taken to a volumetric flask of 20 mL using the same methodology. This procedure was prepared in duplicate for the dosing and control standards solutions. For the solvents with immiscible behavior with the solvent methanol: water (50:25, v/v) it was only used pure methanol. The final concentrations was 45 µg/mL.

1.2) Drug content assay

0.5 g of the final gel formulation was taken in a 50 mL volumetric flask and dissolved within methanol:water (50:25, v/v). The solution was sonicated during 5 minutes until complete solubilization and, after that, 1 mL was withdrawn to a 10 mL volumetric flask and then, made up with the solvent. The sample was analyzed by HPLC under the same conditions described on point 1.3.

1.3) Equipment conditions

A Hitachi Elite Lachrom System (VWR, USA) equipped with four Pumps L-2130, an autosampler L-2200, a column oven L-2300, an UV Detector L-2400 and a software EZ Chrom Elite Version 3.2.1. were used in all chromatographic analysis. An analytical GraceSmart RP 18 (250 x 4.6 nm, 5µm) was used.

The method used a mobile phase with 50% (v/v) methanol and 50% (v/v) water. A flow rate of 1.5 mL/min was used with a 15 µL injection volume. The auto sampler chamber was maintained at 16 ºC and oven at 22 ºC. The eluted peaks were monitored at emission wavelengths of 254 nm. The run time was 10 min.

2) Measurement of pH

The pH lecture was determined at room temperature (20 - 25ºC), in triplicate, and after the equipment stabilization. The potentiometer for the oleogel measurements was inoLab® pH 730 and for the hydrogel was Seveneasy®.

3) Apparent viscosity measurement

Shear rate against shear stress measurements were obtained at 20-25ºC using a DV-II + Pro Brookfield® viscometer equipped with spindle nº 65 at 24,47 sec^{-1} (20 rpm), after 30 seconds at room temperature (20 - 25ºC). Continuous flow measurements were performed by increasing the shear rate from 0.36 to 36.71 s^{-1}. Shear stress was measured.

2.3.1. Oleogel

As a reference, a formulation described as “topical pharmaceutical composition comprising flurbiprofen” (patent US 2013/0143831 A1 [86]) was formulated in order to compare the results with the innovator formula. This patent referencing the Froben ® gel marketed by Abbott©. The following formula was adopted for flurbiprofen gel formulation:

Table 9: Formula of Flurbiprofen gel.

Ingredient	% (w/w)	Function
Ethoxydiglycol (Transcutol® P)	64.45	Solvent
Butylated Hydroxytoluene (BHT)	0.05	Anti-oxidant
Sodium Flurbiprofen	5.00	Active ingredient
Hydroxypropyl celullose	1.50	Gelling agent
Propylene Glycol Diperlagonate (DPPG® CG)	24.00	Emollient
PEG-8-Caprylic Capric Glycerides (Labrasol®)	5.00	Co-solvent
Lactic acid 10%	q.s.	pH adjuster

2.3.2. Innovator formula (hydrogel)

2.3.2.1. Solubility studies

Sodium flurbiprofen was added to several solvents or mixtures of solvents: phosphate buffer pH 7.4; Labrasol®; Transcutol® P; DPPG® CG; isopropyl myristate; propylene glycol until saturation. Saturation was achieved when excess solid persisted for more than 12 h with a constant shaking at 20-25ºC. Flurbiprofen was added to a series of propylene glycol – ethanol mixtures varying from 100% of ethanol to 70% of the glycol and stirred at 20-25ºC during 24h.

After ensuring that the solute-solvent equilibrium had been reached, the solutions were centrifuged (Z 400K®, HERMLE, Germany) at 5000 rpm during 10 min and the supernatant solution diluted with methanol:water (50:25) and analyzed by HPLC.

2.3.2.2. Viscosity studies

2.3.2.2.1. Selection of HPC amount

Five gels differing in the amount of hydroxypropyl cellulose (HPC) were prepared in highly purified water to study the quantity of polymer required to obtain the desired viscosity value for the hydrogel formulation. The following concentrations of HPC were prepared: 1.25, 1.5, 2.0, 2.5 and 3.0%. The HPC gels were prepared by dispersing the gelling agent in water under agitation overnight. After this time, the solutions were placed in an ultrasounds bath (8210 Branson®) during 10 minutes. The viscosities were measured in viscometer (Brookfield® Engineering Laboratory, USA) with a spindle nº 65 with a rotation speed of 24.47 sec^{-1} rpm at room temperature (20 – 25ºC).

2.3.2.2.2. Viscosity of marketed gels

To choose an adequate spindle and respectively speed rate, the Emulgel® and Emulgelex® marketed gels from Novartis™ were measured at spindle nº 65, at a rotation speed of 24.47 sec^{-1} rpm at room temperature (20 - 25ºC). The solutions prepared in the previous point 2.3.2.2.1. Were analyzed at the same conditions that marketed gels and the most similar profile were selected for the optimum concentration polymer.

2.2.2.3. Identification of Quality Target Product Profile and Critical Quality Attributes

A QTPP approach was take in product development. This should include some of the most relevant product characteristics depending on the targeted aims and must depend on previous work. From that, potential CQAs could be defined in order to be studied and controlled; CQAs could be derived from QTPP and/or prior knowledge [87] [88] [84].

Table 10: QTPP applied to semisolid formulations (Adapted from: [89])

Elements	Target
Dosage form	Hydrogel
Route of administration	Topical
Dosage	5 % (w/w)
Stability	Stable at 6 or more months at 40 ± 2 ºC

The QTPP characteristics were completed with available information present on followed patent.

CQAs identification are normally associated with drug substance, excipients, and drug product, and the following elements were taken into account to the target product [90] [88] [91]:

Table 11: CQAs applied to the drug product (Adapted from: [90])

Drug product quality attributes		Target
Physical attributes	Rheological behavior	Conform to Ph. Eur. 7.0-2.2.10.
	pH	Conform to Ph. Eur. 7.0-2.2.3.
Identification		Positive for drug substance
Assay		90-110% of flurbiprofen
Tube	Tube homogeneity and uniformity	Conform to USP <3>
In vitro release		Match reference listed drug product

After pre-define the desired QTPP, an Ishikawa diagram was developed to categorize the potential causes of noncompliance. The use of an Ishikawa diagram allows to identify potential risk factors during formulation development and process. With the information used

in DoE experiment and previous knowledge, an Ishikawa diagram was constructed to identify potential risks.

2.2.2.4. Selection of excipients

2.2.2.4.1. Solvents and co-solvents

Ethanol/PG systems were studied as possible solvents for the hydrogel due their high solubility. The remaining sobrenadant of centrifuged samples were analyzed and the pH measured. pH values were adjusted to about 7 with lactic acid 90% and the volume (mL) of acid used in each system registered. The pH was re-measured and registered. Finally, water was added into the system until sodium flurbiprofen precipitation. All solvent percentages were determinate to optimize the solvent amounts to the formulation. All measures were performed at room temperature (22 - 25ºC).

2.2.2.4.2. Permeation enhancers (Oleic Acid and Menthol)

2.2.2.4.2.1. Design of experiments approach

In order to study the optimum concentration of the used permeation enhancers (Acid oleic and menthol), it was considered a range for each one of the literature; for oleic acid the concentrations were 0.5 [92] – 3% [93] and for menthol 1 – 5% [94], minimum and maximum, respectively. The formula of the gels was optimized and the independent variables were oleic acid and menthol concentration.

This design required 7 experimental runs, including three replicated center points for a more uniform estimation of the prediction variance over the entire design space. Data were analyzed using the MODDE® Pro 11 software (Umetrics, Sweden) and effects were considered significant when the estimated p values were lower than 0.5 (the chosen alfa error), to increase statistical power. The following mathematical quadratic model was fitted to the data:

Equation 8: $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_{12}$

This model describes the zero and second order effects as well as the interactions between the independent variables. In this equation, β_0 is the arithmetic mean response, β_1, β_2, and β_{11} are the linear and quadratic coefficients of the independent variables and β_{12} the interaction term, respectively. The higher the magnitude of each coefficient, the higher is the respective main effect on the system. A positive coefficient sign indicates that an increase in the parameter level leads to an increase in enhancer concentration. Taking into account the

interaction coefficient, the response must be studied in terms of how the variation of one factor modulates the effect of another factor (Table 12).

Table 12: Formula matrix and experimental matrix.

Design matrix		ID	Experimental matrix	
Oleic acid (%)	**Menthol (%)**		**Oleic acid (%)**	**Menthol (%)**
-1	-1	Run 7	0.5	1
1	-1	Run 3	3	1
1-	1	Run 2	0.5	5
1	1	Run 4	3	5
0	0	Run 6	1.75	3
0	0	Run 5	1.75	3
0	0	Run 1	1.75	3

A second DoE was performed using the same methodology but varying the factors range:

Table 13: Formula matrix and experimental matrix.

Design matrix		ID	Experimental matrix	
Oleic acid (%)	**Menthol (%)**		**Oleic acid (%)**	**Menthol (%)**
-1	-1	Run 6	0.099	0.25
1	-1	Run 2	2.5	0.25
1-	1	Run 5	0.099	3
1	1	Run 4	2.5	3
0	0	Run 3	1.3	1.625
0	0	Run 1	1.3	1.625
0	0	Run 7	1.3	1.625

PEG-40 Hydrogenated Castor Oil (Tagat® CH 40) was used as a solubilizing agent of the oleic acid in the aqueous formulation and her final concentration was determined by constant addition until a full enhancer solubilization.

2.2.2.4.2.2. *In vitro* release

The *in vitro* permeation of sodium flurbiprofen was performed according to guideline OCDE 428 [77]. Silicone membranes were cut and submersed into phosphate buffer solution (PBS) at pH 7.4 during 30 minutes and then mounted between the donor and receiver

compartments on static vertical Franz diffusion cells (receptor volume: 3 mL, permeation area: 1 cm^2. PBS was used as receptor phase to assure perfect *sink conditions* in the whole experiment. It was constantly stirred with a small magnetic bar (200 rpm) and thermostated at 32 ± 0.5 °C throughout the experiments. The samples were then applied (0.2 to 0.4 g) evenly on the surface of the membrane in the donor compartment and sealed by Parafilm® immediately to prevent water evaporation. Samples were collected from the receptor fluid at pre-determined time points: 2, 4, 6, 8, 10, 12 and 24 hours and replaced with an equivalent amount (200 µL) of receptor medium. The drug content in the withdrawn samples was analyzed by HPLC. Repeated measures, using at least 3 replicated cells for each formulation, were used.

2.3.3. Statistical analysis

Data was presented in mean ± standard deviation (mean ± SD). Microsoft® Excel, version 2013, was used to analyze statistical data. The statistical evaluation of data was realized by analysis of variation (ANOVA) using regression analysis and considering a p-value of 0.05 as a minimal level of significance. XLSTAT® version 2013 was used to regression analysis and determinate AIC values.

2.4. Results

2.3.1. Innovator formula (hydrogel)

2.3.1.1. Viscosity studies

Water – HPC gels at different concentrations (1.25, 1.50, 2.0, 2.5 and 3.0% of HPC) were prepared and viscosity was assessed as well as for oleogel and Emulgel® and Emulgelex® marketed gels. Figure 7 shows the representative flow curves (shear stress function of shear rate) with apparent viscosity values calculated at the apex of the loop:

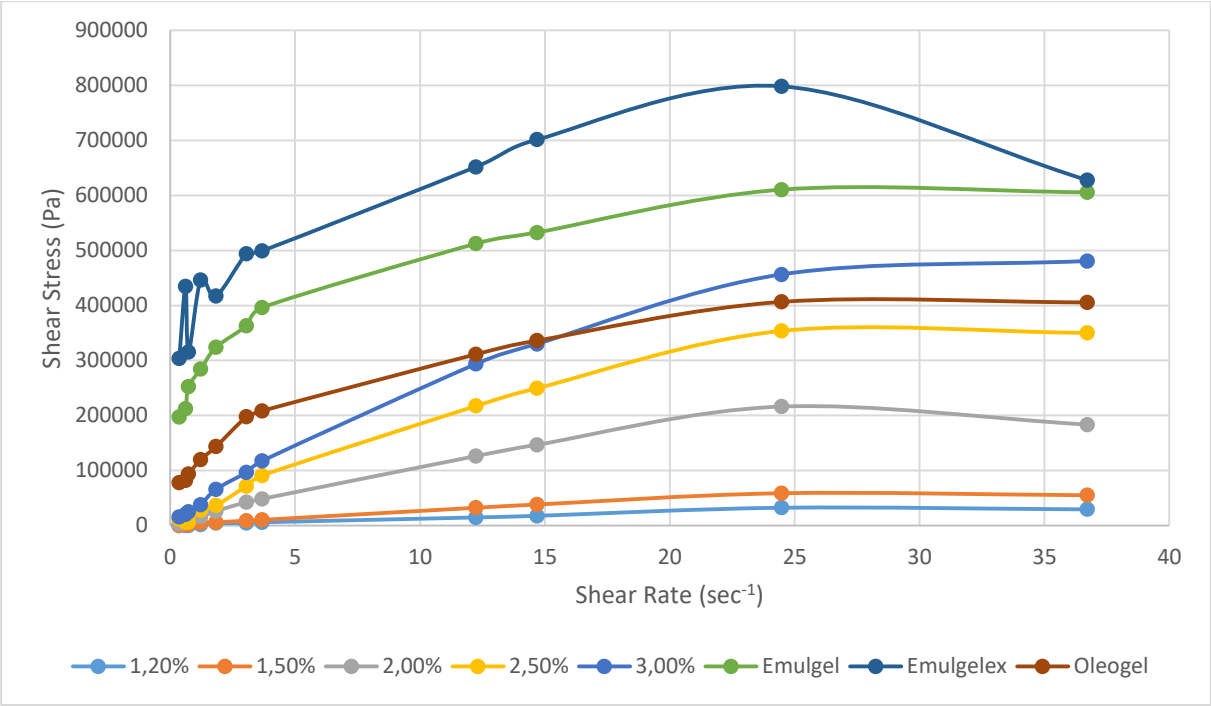


Figure 7: Typical flow curve of shear stress as function of shear rate for water-HPC gels and Emulgel®, Emulgelex® and oleogel formulations.

All formulations present the same rheological profile once that the relation between shear stress and the shear rate is not a constant. The results show that apparent viscosity decreases with the increase in shear rate (Figure 7). All formulations present a non-Newtonian profile, more precisely, a pseudoplastic or shear thinning behavior, ideally for topical applications. Pseudoplastic behavior are a non-Newtonian system once that viscosity are not a constant when shear rate increase. Instead of that, pseudoplastic systems decreases viscosity when shear rate increases. For that, topical formulations are easier to spread through skin [95].

Apparent viscosity values were determined for all formulations in the maximum shear stress (at 24.47 s^{-1}). The values for each formulation are listed in table 14. The maximum of HPC concentration recommended by the patent it is 5% of HPC [86] (more preferably up to 3%) and apparent viscosity of 25,000 mPa.s (between 20,000 – 40,000 mPa.s) (Table 14):

Table 14: Apparent viscosities of the water – HPC gels and Emulgel® and Emulgelex® in order to study gels viscosities.

Sample	Apparent Viscosity (mPa.s) at $24.47s^{-1}$
Gel 1 (1.25%)	1320
Gel 2 (1.50%)	2399
Gel 3 (2.0%)	8838
Gel 4 (2.5%)	14457
Gel 5 (3.0%)	18656
Emulgel®	24955
Emulgelex®	32633
Oleogel	16616

An oleogel preparation according to the description on the patent was used to, in order to evaluate the real apparent viscosity. Oleogel preparation present a closely apparent viscosity to gel 4: 14457 mPa.s and 16616 mPa.s to gel 4 and oleogel, respectively.

2.3.1.2. Quality Target Product Profile and Critical Quality Attributes

The most important element formulation design is pre-defining a QTPP. Some factors could influence stability on final formulation. In order to identify variables that may have an impact on final formulation, an Ishikawa diagram was developed. The CQAs were identified and represented on an Ishikawa diagram (Figure 8):

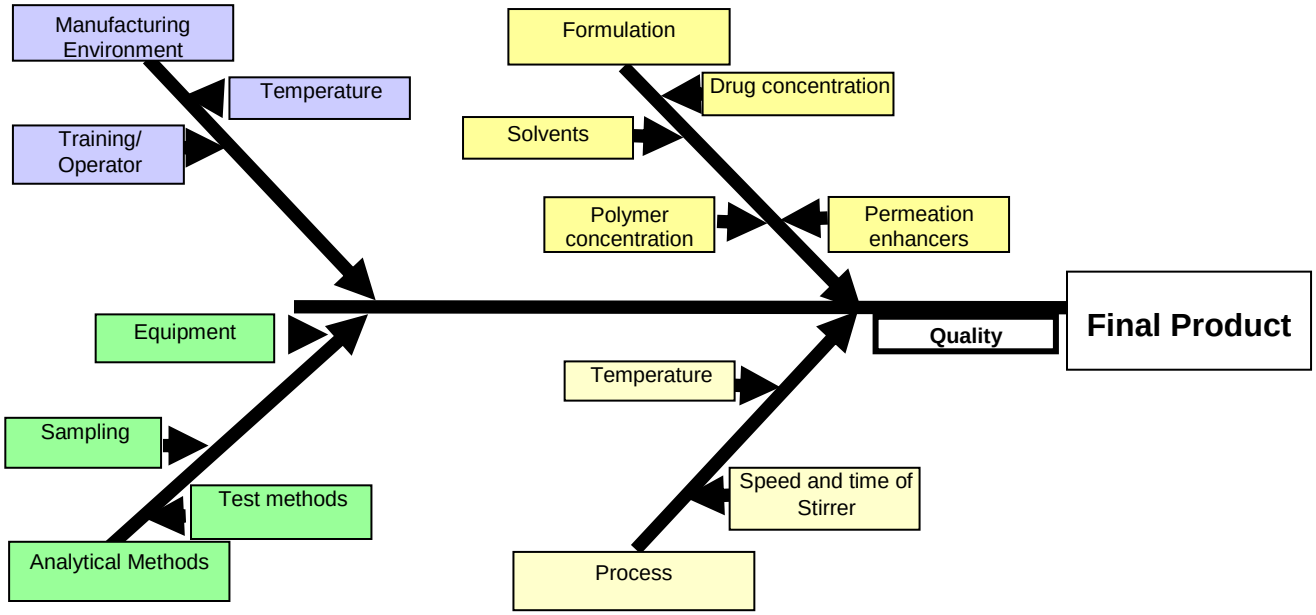


Figure 8: Ishikawa diagram illustrating factors that may have impact on hydrogel preparation.

The physicochemical characterization and *in vitro* efficacy of the final formulation could depend of some variables such as polymer and enhancer concentration, respectively. Since that in the present study, the main goal is the achieving of a final stable flurbiprofen gel formulation, it is crucial to identify possible variables that could influence the permeation of the drug. The permeation enhancers concentration would be studied with a DoE approach.

2.3.1.3. Solubility tests

The solubility of sodium flurbiprofen in different solvents was investigated. Once flurbiprofen has a low solubility in water (0.008 mg/mL), flurbiprofen sodium salt was studied due to its potential to increase solubility in solvents relatively to flurbiprofen [96]. It was observed (Table 15) that flurbiprofen sodium has a higher solubility on propylene glycol (406.4 ± 6.58 mg/mL) and also, on systems combined with ethanol, where PG exists in a higher percentage. Flurbiprofen sodium also exhibits higher solubility on Transcutol® P (262.91 ± 5.32 mg/mL).

Table 15: Flurbiprofen solubilities in different solvents (mean ± SD, n=3)

Solvents	Solubility ± SD, n=3 (mg/mL)
Phosphate buffer pH 7.4	19.66 ± 0.04
Transcutol® P	262.91 ± 5.32
Propylene glycol	406.4 ± 6.58
DPPG® CG	0.32 ± 0.08
Isopropyl myristate	0.13 ± 0.03
Labrasol®	162.98 ± 2.89
Ethanol/PG (100%/0%) - 1	104.21 ± 0.86
Ethanol/PG (70%/30%) – 2	297.64 ± 13.48
Ethanol/PG (50%/50%) – 3	343.04 ± 7.05
Ethanol/PG (30%/70%) – 4	372.1 ± 14.44

The use of PG is considered to be nontoxic and shows a low irritancy *in vivo* studies, moreover, has a humectant and permeation function, fundamental on a topical formulation. The use of PG associated with ethanol (acting as a preservative) seems to be a suitable choice to a mixture system solvent [35]. It was observed that when the percentage of ethanol increases and the PG increases, the solubility of flurbiprofen decrease (Figure 9):

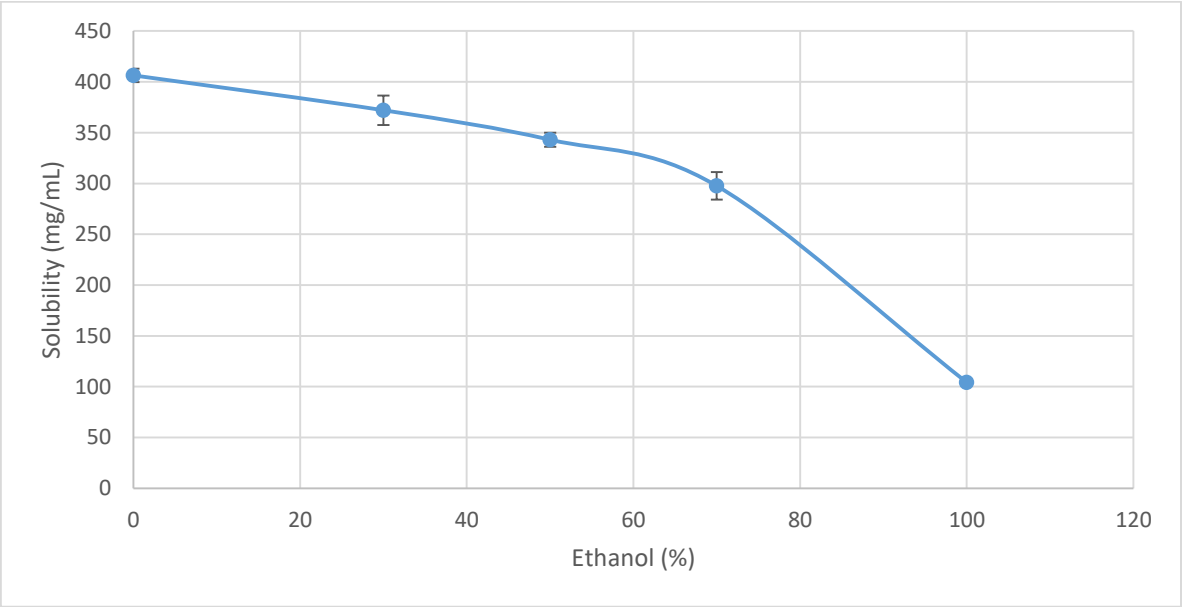


Figure 9: Solvent solubility in different % of ethanol/PG mixtures (mean ± SD, n=2)

Ethanol was previously used at 20% and, combined with PG, enhancing the permeability of the gels [60].

2.3.1.4. Selection of excipients

2.3.1.4.1. Solvents and co-solvents

The ethanol/PG system mixtures were evaluated. The maximum percentages of water were determined once that are no references on oleogel patent about water content. The assay revealed an increase in water solubility when ethanol quantity decreases, except for the system 4 (ethanol at 30% and PG at 70%). System 3 revealed a higher flurbiprofen water solubility (Table 16):

Table 16: Solvents concentrations to the hydrogel formulation (mean ± SD, n=2)

	Sample (mean ± SD, n=3)			
	1	**2**	**3**	**4**
Water added (%)	33.7 ± 1.20	45.5 ± 3.42	45.7 ± 3.10	34.0 ± 2.26
PG/Ethanol (%)	58.50 ± 2.7	48.20 ± 3.3	48.20 ± 3.0	58.90± 2.0
Lactic acid (%)	2.80 ± 0.73	1.30 ± 0.09	1.20 ± 0.10	2.10 ± 0.28
Flurbiprofen (%)	5	5	5	5

Table 16 describes the total amount of each excipient in each system (which includes water, PG, ethanol, lactic acid and flurbiprofen – 100%). Like previously cited, sodium flurbiprofen got a higher solubility on systems with lower ethanol contents and higher of propylene glycol. Ethanol amount was defined to 20% according to what was previously referred. Propylene glycol quantity was determined according to previous results: once that 20% of ethanol was defined to the formulation, this value was withdrawn from the final percentage of the PG/ethanol system. So, the average of PG was 33.5% in the formulation (defined to 34%).

2.3.1.4.2. Permeation enhancers (Oleic Acid and Menthol)

To determinate the best permeation enhancers concentration, a QbD approach was developed. Oleic acid and menthol were previously mentioned in topical anti-inflammatory non-steroid formulations. Oleic acid is used due to lipid disrupting properties, increasing the fluidity of SC lipids, and menthol once that usually decrease the lag time for permeation [94]. Although a large range of permeation enhancers concentrations are cited by literature, only the most used were considered. For that, a range of 0.5 – 3% and 1 – 5% for oleic acid and menthol, respectively, were included in this study. MOODE 11® (Umetrics™) was used in order to

design the following screening: oleic acid and menthol (factors), permeation (response) and a full factorial design (2 levels – 7 runs). In order to define a target value, an *in vitro* study for the oleogel in silicone membranes was performed. The samples for each run were analyzed in terms of pH values and drug recovery:

Table 17: Results from pH and drug content to DoE screening formulations.

Sample	Drug Recovery (%) (mean ± SD, n=3)	pH
Oleogel	89.09 ± 2.22	7.16
Run 1	82.67 ± 4.31	6.86
Run 2	88.02 ± 1.26	6.22
Run 3	100.9 ± 14.16	6.72
Run 4	88.50 ± 11.57	6.90
Run 5	89.03 ± 8.24	7.03
Run 6	88.81 ± 6.81	7.01
Run 7	85.21 ± 1.29	6.23

Figures 10 and 11 show the permeation profile for oleogel and hydrogel runs through silicone membrane, presenting the cumulative amount of flurbiprofen released in function of time, during 4 hours:

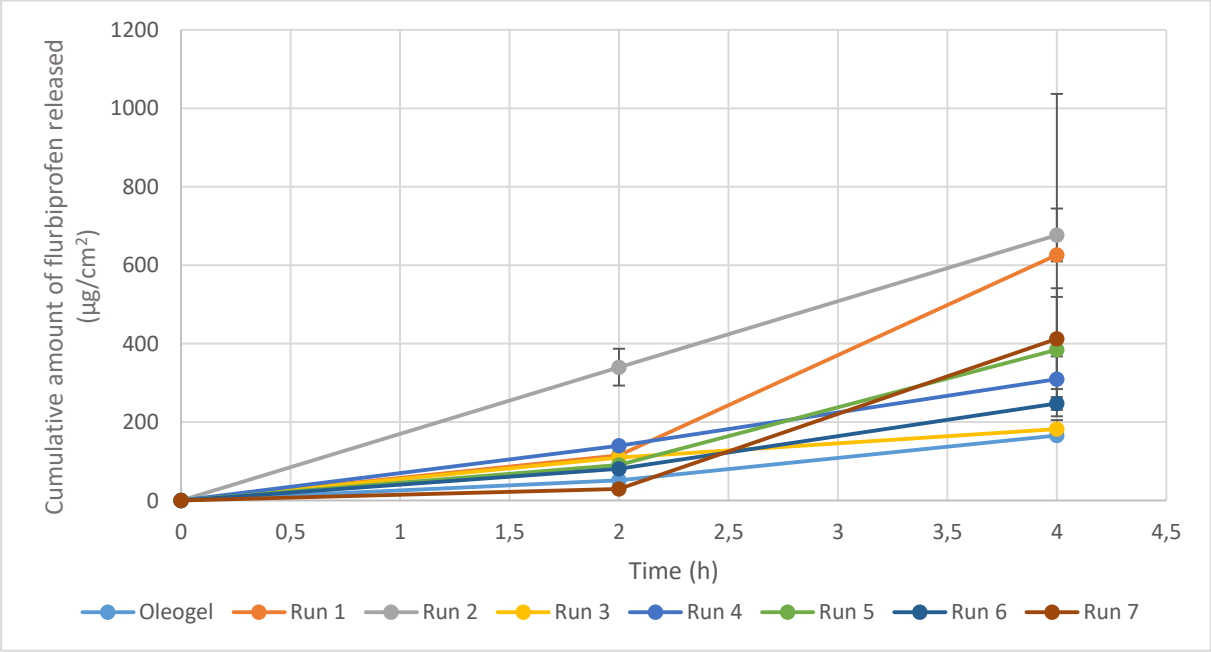


Figure 10: Release profile of flurbiprofen (4h) for oleo and hydrogels through silicone membrane represented in µg/cm² (mean ± SD, n=3)

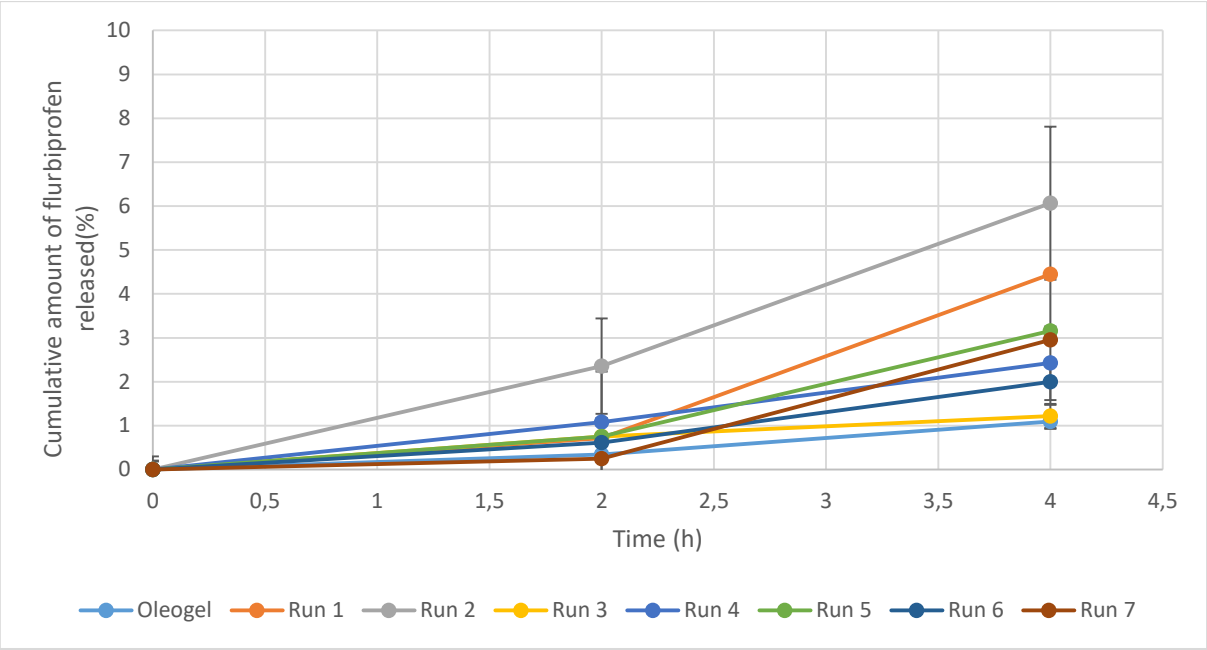


Figure 11: Release profile of flurbiprofen (4h) for oleo and hydrogels through silicone membrane represented in % (mean ± SD, n=3)

The permeation was quantified for each run for the oleogel, and the value for oleogel was considerate the target value for the screening:

Table 18: Values for *in vitro* release (4h) in silicone membrane for DoE screening (mean ± SD, n=3).

Run	Oleic Acid (%)	Menthol (%)	Release % (mean ± SD, n=3)
1	1.75	3	4.45 ± 2.64
2	0.5	5	6.07 ± 1.09
3	3.0	1	1.22 ± 0.42
4	3.0	5	2.42 ± 0.79
5	1.75	3	3.16 ± 1.48
6	1.75	3	2.0 ± 0.04
7	0.5	1	2.95 ± 0.46
Oleogel	-	-	1.02 ± 0.08

Oleogel permeation percentage was about 1.02%.

ANOVA analysis was performed to analyze the significance of the model from the analyzed data. The model shows a $p > 0.05$ (0.132) to the regression model which suggest a not

significant model. The adequacy of the developed model was estimated by the lack of fit and the R^2, with a value of 0.972 and 0.810, respectively, suggesting that model has no lack of fit ($p > 0.05$) but the probability of regression is not significant at 95%, revealing a model statistically poor. The lack of fit estimates the error variance independently of the model and a lack of fit superior to alfa error indicates that variability measured by replicates does not explain the gap between predicted and experimental points. The results of regression analysis were expressed in scaled and centered coefficients, increasing the interpretability of the model, practice very common in DoE experiments. The regression analysis results are shown in table 19:

Table 19: Summary of regression analysis results for permeation enhancers screening.

Term	Coefficients (Scaled and Centered)	Standard Error	*p*
k	1.930	0.046	0.0035
Oleic Acid	0.368	0.060	0.0744
Menthol	0.031	0.060	0.1195
Oleic Acid*Menthol	0.204	0.060	0.4078

*k – constant; p – p value

The terms did not reveal any statistical significance once their $p > 0.05$. None of the runs permeation achieves the target value of oleogel permeation. The runs with a closer permeation value of oleogel were considered, and the one who revealed a closer value was the run 3, containing 3% of oleic acid and 1% of menthol. A new range of permeation enhancers concentrations was defined: 0.2 – 2.5% to menthol and 0.25 – 3% to oleic acid in order to decrease permeation value to achieve the target value. A second DoE was created and the values for release analyzed:

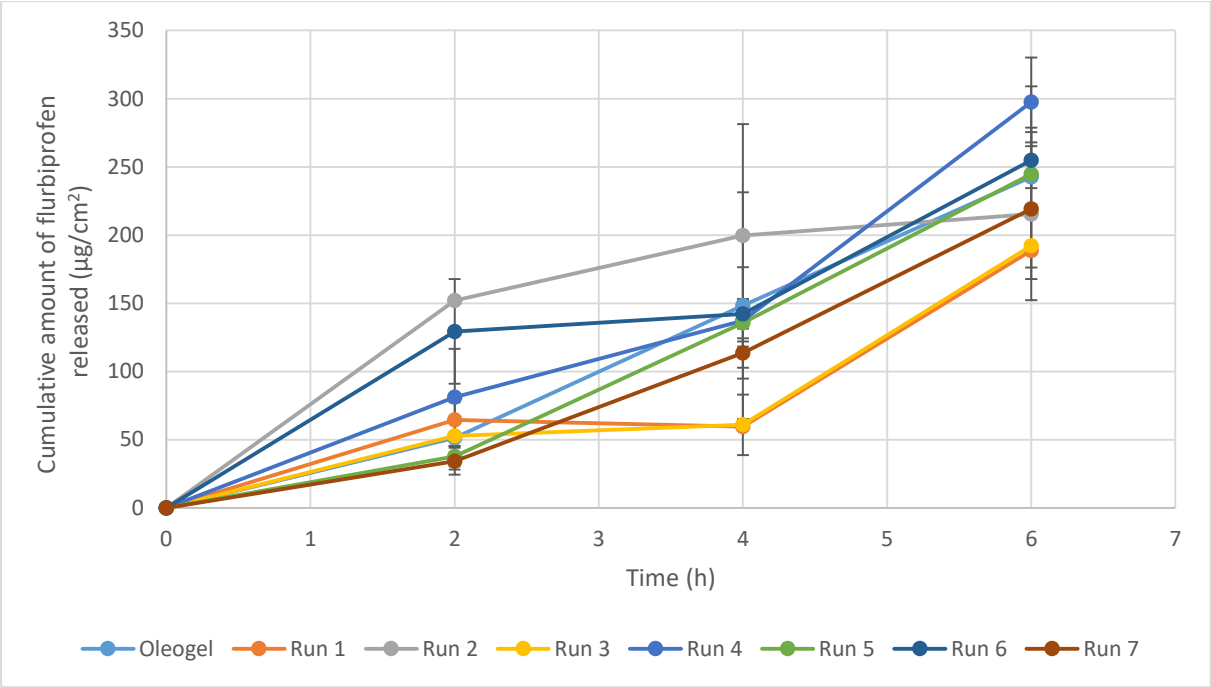


Figure 12: Release profile of flurbiprofen (6h) for oleo and hydrogel through silicone membrane represented in µg/cm² (mean ± SD, n=3)

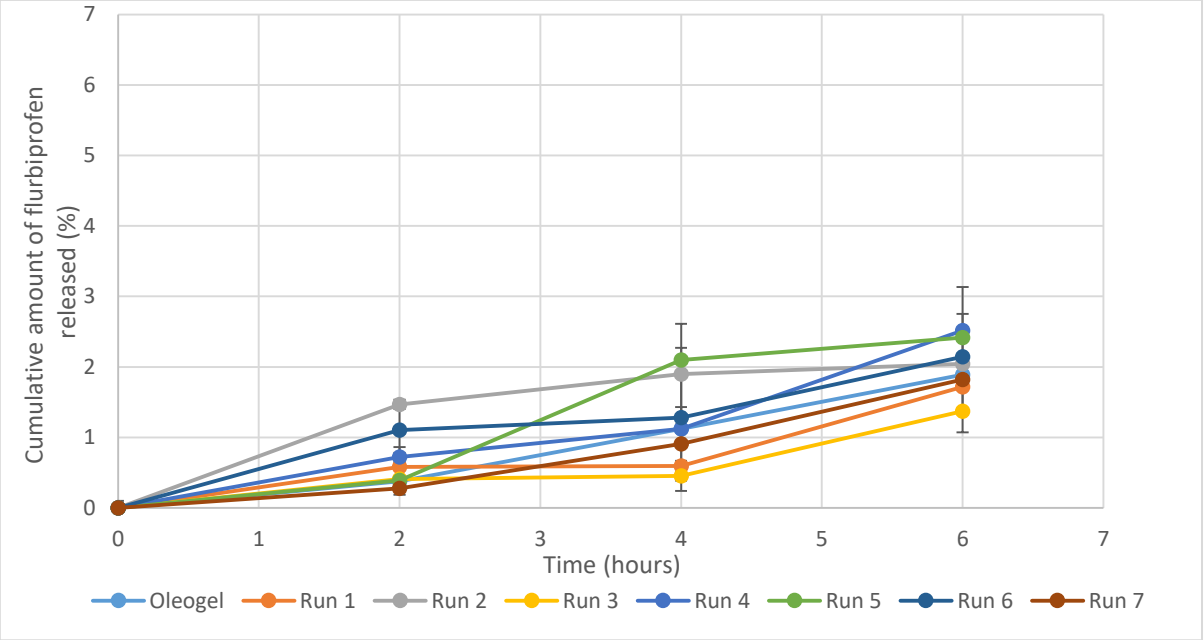


Figure 13: Release profile of flurbiprofen (6h) for oleo and hydrogel through silicone membrane represented in % (mean ± SD, n=3)

Figures 12 and 13 presents the permeation profile of flurbiprofen through 6 hours of experiment, cumulative amount of flurbiprofen released in function of time.

Table 20: Values for *in vitro* permeation (n=6h) in silicone membrane for DoE screening (mean ± SD, n=3).

Run	Oleic acid (%)	Menthol (%)	Release % (mean ± SD, n=3)
1	1.3	1.625	1.82 ± 0.37
2	2.5	0.25	2.04 ± 0.71
3	1.3	1.625	2.10 ± 0.00
4	2.5	3.0	2.51 ± 0.56
5	0.099	3.0	1.37 ± 0.30
6	0.099	0.25	1.72 ± 0.08
7	1.3	1.625	1.96 ± 0.37
Oleogel	-	-	1.89 ± 0.62

According to regression analysis of the model, all coefficients, and standard errors were analyzed and oleic acid enhancer revealed a $p > 0.05$, being no statistically significant. However, the interaction with oleic acid and menthol revealed a significant interaction ($p < 0.05$). The model also exhibits a suitable R^2 and Q^2 with values of 0.942 and 0.724. Overall, the model seems to be suitable to the experimental data (Table 21).

Table 21: Summary of regression analysis results for permeation enhancers screening.

Term	Coefficients (Scaled and Centered)	Standard Error	p
k	3.181	0.3779	2.88×10^{-5}
Menthol	-1.345	0.4999	0.009
Oleic Acid	1.08	0.4999	0.642
Oleic Acid*Menthol	-0.48	0.4999	0.043

Concerning regression analysis, the model was statistically significant as $p < 0.05$ (0.023) for the permeation response. The suitability of the fit was estimated by the lack of fit and a value of 0.695 was obtained, presenting no lack of fit by the model ($p > 0.05$).

In order to describe the kinetics of flurbiprofen release through the experiment, data were fitted to zero order, first order and Higuchi models.

Table 22: Kinetic parameters obtained after fitting the release data from oleogel formulation and DoE experiments (mean ± SD, n=6)

		Model		
Formulation		**Zero-order**	**First-order**	**Higuchi**
Run 1	R^2	0.82 ± 0.12	0.81 ± 0.02	0.86 ± 0.04
	K	-2.58 ± 5.40	0.99 ± 0.10	0.97 ± 20.50
	Q_0	18.74 ± 13.50	0.84 ± 0.07	82.29 ± 34.23
	AIC	13.87 ± 20.12	3.22 ± 0.95	30.66 ± 3.22
Run 2	R^2	0.82 ± 0.12	0.75 ± 0.04	0.857 ± 0.02
	K	-2.59 ± 5.40	1.01 ± 0.03	-7.04 ± 4.61
	Q_0	18.74 ± 13.50	0.78 ± 0.00	27.93 ± 27.67
	AIC	13.87 ± 20.12	3.89 ± 0.8	6.90 ± 20.76
Run 3	R^2	0.80 ± 0.06	0.70 ± 0.05	0.81 ± 0.04
	K	19.13 ± 18.70	1.28 ± 0.22	-2.24 ± 7.21
	Q_0	33.91 ± 9.77	0.80 ± 0.05	83.97 ± 27.09
	AIC	25.74 ± 7.50	5.23 ± 1.38	32.29 ± 2.34
Run 4	R^2	0.83 ± 0.10	0.81 ± 0.09	0.77 ± 0.14
	K	-11.30 ± 12.16	0.88 ± 0.27	-12.70 ± 3.36
	Q_0	29.34 ± 2.64	0.80 ± 0.02	65.19 ± 5.40
	AIC	29.12 ± 5.9	2.11 ± 2.8	30.97 ± 4.18
Run 5	R^2	0.804 ± 0.14	0.81 ± 0.11	0.721 ± 0.06
	K	-9.62 ± 21.34	0.87 ± 0.44	-21.77 ± 16.24
	Q_0	38.63 ± 13.34	0.86 ± 0.09	87.35 ± 26.63
	AIC	32.51 ± 0.17	2.22 ± 3.7	34.78 ± 2.16
Run 6	R^2	0.93 ± 0.02	0.82 ± 0.08	0.81 ± 0.07
	K	-11.65 ± 14.58	0.95 ± 0.19	-23.59 ± 11.21
	Q_0	40.52 ± 6.34	0.87 ± 0.04	91.08 ± 12.98
	AIC	29.11 ± 0.89	2.9 ± 1.74	33.31 ± 2.33
Run 7	R^2	0.88 ± 0.11	0.71 ± 0.05	0.879 ± 0.06
	K	16.34 ± 13.43	1.315 ± 0.125	-5.24 ± 8.9
	Q_0	38.71 ± 0.21	0.84 ± 0.01	93.94 ± 3.5
	AIC	24.96 ± 9.96	5.5 ± 0.8	31.25 ± 2.65
Oleogel	R^2	0.93 ± 0.07	0.87 ± 0.08	0.787 ± 0.09
	K	-15.83 ± 14.22	0.46 ± 0.55	-23.25 ± 11.38
	Q_0	32.54 ± 7.37	0.86 ± 0.07	71.66 ± 13.16

	AIC	27.84 ± 2.48	0.72 ± 2.16	31.61 ± 4.34

. K– Release constant; Q_0 – initial drug concentration; R^2 – Correlation coefficient; AIC - Akaike informational criterion

In order to evaluate the best fit model, two parameters were considered: coefficient of determination, R^2 and Akaike information criterion (AIC), where it is pretended the highest value of R^2 (close to 1) and a lowest AIC value. Three different fit models were used: zero order, first-order release, and Higuchi model. The results show that the model that better fits the obtained results was the first order release model. R^2 was not used as decision factor once that differences between the models were not identified. AIC on first order release was lower comparatively to zero order and Higuchi models [39], [97], [98].

According to figure 14 and 15, it is possible to analyze the surface and contour response to permeation response, varying the factors. The 3D modulation is possible because experimental replicates central point were developed. The results show that an increasing on menthol and oleic acid concentration, there is an increase on permeation too. The flurbiprofen permeation values are represented according to oleic acid and menthol variation:

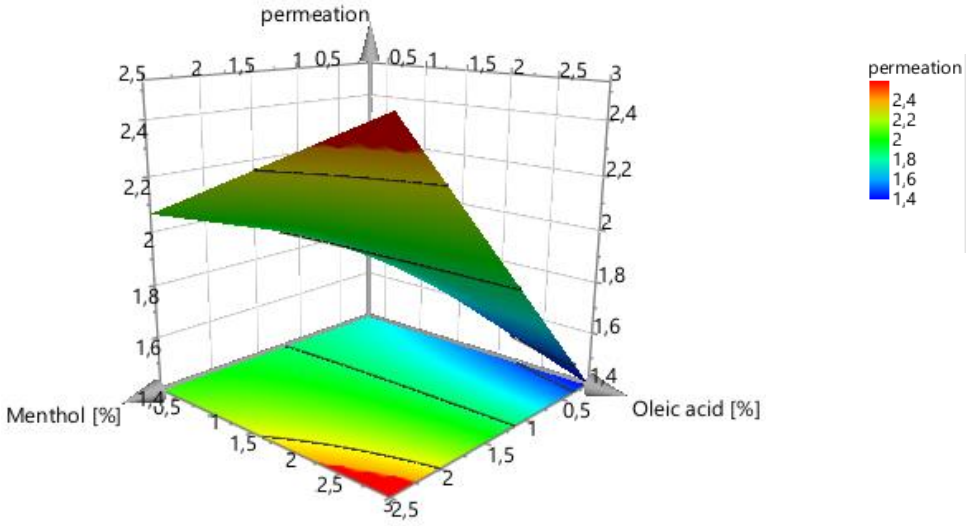


Figure 14: Response surface plot for screening.

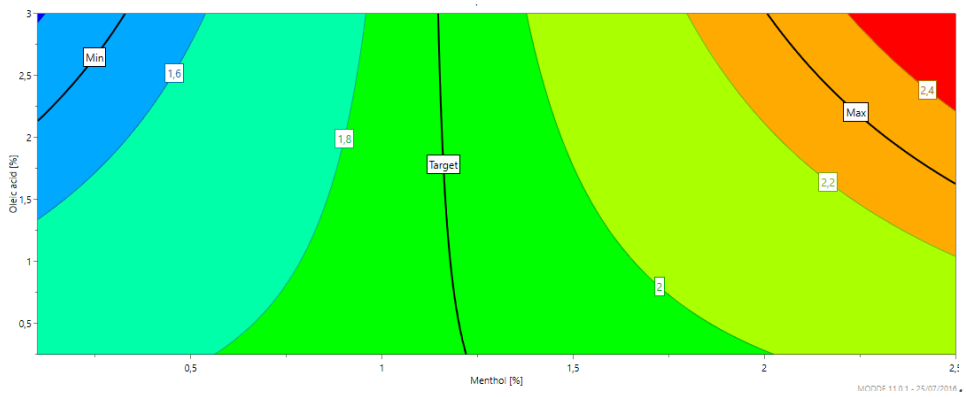
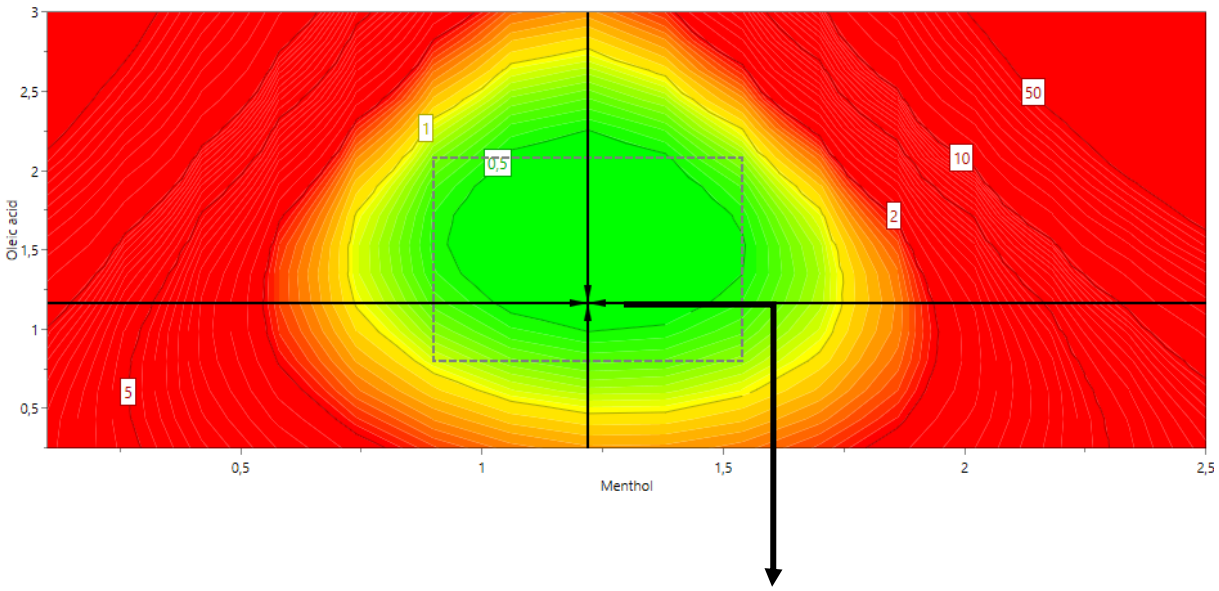


Figure 15: Response contour plot for screening.

Once the design space was determined, an optimization approach was applied. The green area of figure 16 represents the concentration where oleic acid and menthol concentration remains between the acceptable limits for the permeation. The red area represents the area where the permeation will fall out the acceptable concentrations. The furthest away from the center, higher the probability of failure of the product formulation. The set point analysis of the DS determinates an optimal point, where both concentrations are at their best concentration ensuring that all CQA are accomplished.



Factor	Setpoint
Menthol (%)	1.22
Oleic acid (%)	1.17

Figure 16: Design Space contour for screening optimization.

In the design space, the optimal design area is defined by the green area and the dotted frame defines the optimal conditions that can be inserted in the circular area. The optimal permeation concentration enhancers are 1.17 % of oleic acid and 1.22 % of menthol.

2.5. Discussion

The first step prior to formulation is pre-formulation studies. These studies are responsible for study interactions of drug and excipients with the goal of designing an optimum drug delivery system.

In present chapter, it was proposed a hydrogel flurbiprofen formulation, taking as a reference an oleogel formulation described in the patent US 2013/0143831 A1, present in the annex.

Covering the preformulation studies, the first step was to study the apparent viscosity of two marketed anti-inflammatory gels: Emulgel® and Emulgelex®,once that was not available in the Portuguese market any anti-inflammatory gels with the flurbiprofen. Emulgel® and Emulgelex® had viscosity values of 24955 and 32633 mPa.s. Several water – HPC gels (at 1.25, 1.50, 2.0, 2.5 and 3.0% of HPC) were also evaluated in order to achieve a similar viscosity than oleogel. The gel solution of 2.5% of HPC presents the most similar viscosity value (HPC below the recommended by patent – 3%). So, the Emulgel® and Emulgelex® gels were not considered in terms of viscosities dual here higher viscosity comparatively to oleogel.

Due to the poor water flurbiprofen solubility, flurbiprofen salt was used because of its higher solubility. The solubility studies showed higher values in PG (406.4 ± 6.58 mg/mL) and for the Transcutol® P (262.9 ± 5.32 mg/mL). Consequently, PG solvent was used as an excipient of the hydrogel formulation. The ethanol/PG system at different concentrations was also studied, and the ethanol/PG at 30 and 70%, respectively, presented a high flurbiprofen solubility (372.1 ± 14.44 mg/mL). The ethanol was used as co-solvent and as preservative, at a concentration of 20% [60]. PG was defined to 34%.

The zero order, first order and Higuchi model were applied to the previous results, and, in order to predict the goodness of fit of the data, R^2 and AIC parameters were also studied. The first order release model show a better data fit once that R^2 is very similar in all formulations but AIC criteria shows the lowest values for this model.

The permeation enhancers concentrations were also studied, applying a DoE approach. Menthol and oleic acid were used in the present formulation due to their common use on topical applications [99]. Menthol is a terpene often used as penetration enhancer due to his high percutaneous ability, low skin irritancy and low systemic toxicity. Its mechanism is characterized by the disorder of the lipophilic chain on SC, promoting diffusivity of the drug

[72] [100]. Oleic acid is a fatty acid responsible by interfering with SC permeability by disrupting the molecular lipidic matrix [101]. Both permeation enhancers were used in the hydrogel formulation. A range of 0.5 – 3% for oleic acid [92] and 1 – 5 % for menthol [94] was used according literature [102] [103].The first DoE experiment presented a large range of permeation values and the closest from oleogel value was the run with 1% of menthol and 3% of oleic acid. The higher the concentration of menthol and lower the oleic acid, higher the permeation, the second DoE goal was to consider lower permeations. 0.2 – 2.5% and 0.25 – 3% of menthol and oleic acid, in this order, were defined to the new experiment. The decrease of drug release when OA increase concentration, through the experiments. It is explained in the literature by the increase of viscosity in higher concentrations of oleic acid, leading to a poorly flurbiprofen release [104] [105]. In the second DoE, a DS was defined and a concentration of oleic acid and menthol was optimized to lower oleic acid concentration at 1.17% and higher to menthol (1.22%).

3rd Chapter – Product Development

3. Introduction

The aim of this chapter was a hydrogel formulation development taking into account the results obtained in chapter 2. Oleogel formulation (reference) was also developed according to description on the patent. Both formulations were physicochemical characterized and stability studies were assessed. Furthermore, an *in vitro* permeation and retention studies were performed.

3.1. Materials

The materials used are described in the 2nd chapter, section 2.1.

3.2. Equipments

The equipments used are described in 2nd chapter, section 2.2.

3.3. Methodology.

3.3.1. Oleogel

3.3.1.1. Product preparation

In order to prepare the oleogel formulation, the following procedure was performed as described in the patent:

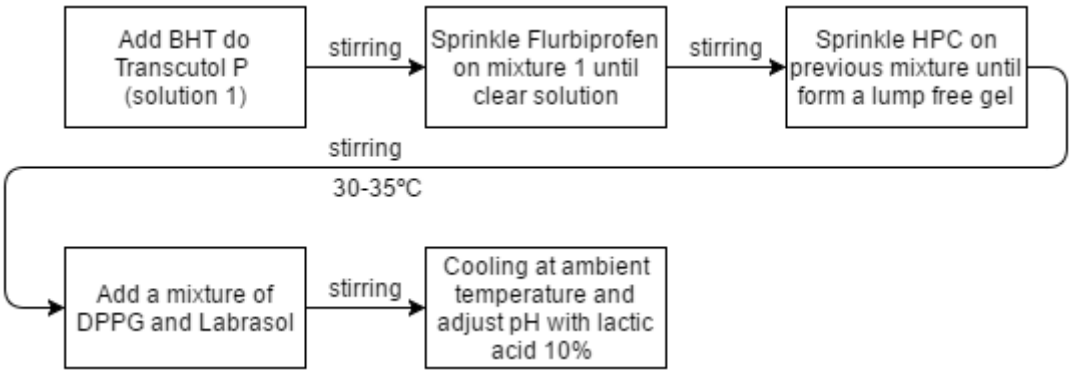


Figure 17: Product process of the oleogel formulation (Adapted from: [86])

There was no reference to any pH adjustment on patent. However, acid lactic was used at 10% to obtain a pH value around 7 [74].

3.3.1.2. Oleogel characterization

The final formulation was characterized in terms of pH values, apparent viscosity and organoleptic characteristics. The methodology to pH and apparent viscosity are described in 2nd chapter, section 2.3.

3.3.2. Hydrogel

3.3.2.1. Hydrogel formulation

The following formulation was used to the product preparation:

Table 23: Qualitative and quantitative composition (%, w/w) of hydrogel formulation.

Ingredient	% (w/w)	Function
Flurbiprofen	5.0	Active ingredient
Ethanol	20.0	Solvent/Preservative
Propylene glycol	34.0	Solvent
HPC	2.5	Polymer
Oleic acid	1.17	Permeation enhancer
Menthol	1.22	Permeation enhancer
Tagat® CH 40	6.0	Solubilizer
Water	30.11	Solvent
Lactic acid 90%	Until pH 7	pH adjuster

3.3.2.2. Product preparation

In order to minimize possible errors due to preparation deviations in both formulations, the same procedure was adopted (Figure 18):

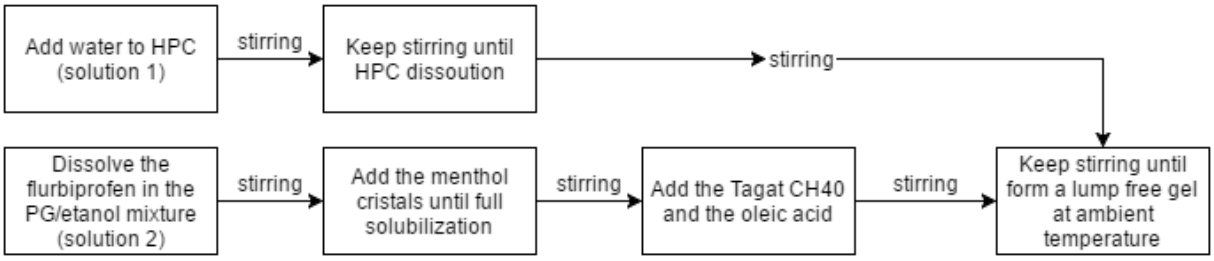


Figure 18: Product preparation for hydrogel formulation

Sodium flurbiprofen was dissolved in the main solvent system (PG/Ethanol) (solution 2). The solution 1 corresponds the intumescence of HPC in water. The permeation enhancers were added to the solution 2. Then gel was added to the solution 2. The gel obtained was maintained under agitation (300 rpm) until achieving a homogeneous gel.

3.3.3. *In vitro* skin permeation

The skin permeation of flurbiprofen was measured using Franz diffusion cells and newborn pig skin obtained from a local slaughterhouse. The entire skin was cut into sections (1 cm^2 permeation area). PBS was used as the receptor phase that assured perfect sink conditions during all experiment period. The cells were immersed in a bath system at 32 ± 0.5 °C under stirring (200 rpm). The formulations samples were applied (0.3 ± 0.1 g, infinite dose experiment) on the skin surface in the donor compartment further sealed by Parafilm® in order to prevent the water evaporation (occlusive conditions). Samples were collected from the receptor fluid at pre-determined time points 2, 4, 6, 8, 10, 12 and 24 h and replaced with an equivalent amount (200 µl) of fresh receptor medium. The flurbiprofen content in the withdrawn samples was determined by HPLC as described before.

3.3.4. *In vitro* skin retention

In vitro skin retention study was performed by tape stripping according to the method recommended by OECD Guideline 428. The samples for oleo and hydrogel (0.3 ± 0.1 g) were spread over the newborn pig skin (1 cm^2) in contact with ± 4 ml of receptor phase as described before. After 24 h, skin samples were rinsed to remove the excess of formulation and dried with filter paper. After the skin samples had been attached and fixed on a smooth surface, the SC was removed using 20 adhesive tapes (Scotch® 3M,UK). In order to ensure the reproducibility of the tape stripping technique, a cylinder (2 kg) on foam and an acrylic disk were used and the pressure was applied for 20 s for each tape. All the tapes (excluding the first one) with the removed SC and the remaining skin (viable epidermis and dermis - ED) were

cut into small pieces used for the extraction process previously validated. In this extraction process, 3 ml of ethanol was added to the SC tapes and ED pieces. Both samples were vigorously stirred for 2 minutes in a vertical mixer (Kinematica AG) and sonicated for 20 min to lyse cells. The final solution was centrifuged (30000 rpm, 10 min) and the supernatant was filtered (0.2 µm) and assayed as above described to quantify the amount (%) of flurbiprofen retained in these skin layers (SC + ED).

3.3.5. Stability studies

Hydrogel and oleogel formulations were stored during 4 weeks at 25ºC and 60% of relative humidity (RH), 30ºC and 65% of RH and 40ºC and 75% of RH, protected from light in aluminum tubes. pH, drug content and organoleptic characteristics were assessed in all formulations.

3.3.6. Statistical analysis

The method are described on 2nd chapter, section 2.3.3.

3.4. Results

3.4.1. Oleogel

3.4.1.1. Oleogel characterization

The physicochemical properties, organoleptic characteristics, viscosity and flurbiprofen content (%) were evaluated on the final formulation of oleogel and the results are presented in table 24:

Table 24: Oleogel physicochemical characterization.

	Value	Specifications
Apparent viscosity (mPa.s)	16616	10.000 – 50.000
pH	7.17	Around 7
Aspect	Conform	Clear and homogenous gel
Odor	Conform	Odorless
Drug recovery (%)	88.9 ± 1.44	90 – 110

3.4.2. Hydrogel

3.4.2.1. Hydrogel characterization

The organoleptic characteristics, apparent viscosity, pH and drug content were evaluated in final hydrogel formulation (Table 25):

Table 25: Hydrogel physicochemical characterization.

	Value	Specifications
Apparent viscosity (mPa.s)	24525	10.000 – 50.000
pH	6.97	Around 7
Aspect	Conform	Clear and homogenous gel
Odor	Conform	Menthol
Drug recovery (%)	89.6 ± 6.0	90 – 110

3.4.3. *In vitro* skin permeation and retention

Permeation profile of flurbiprofen in newborn pig skin was analyzed (Figure 19 and 20). Permeation profiles showed that in both formulations, a similar profile was obtained during 24 hours, however, a decrease on similarity between them, starts to increase after 10 hours ($p < 0.05$):

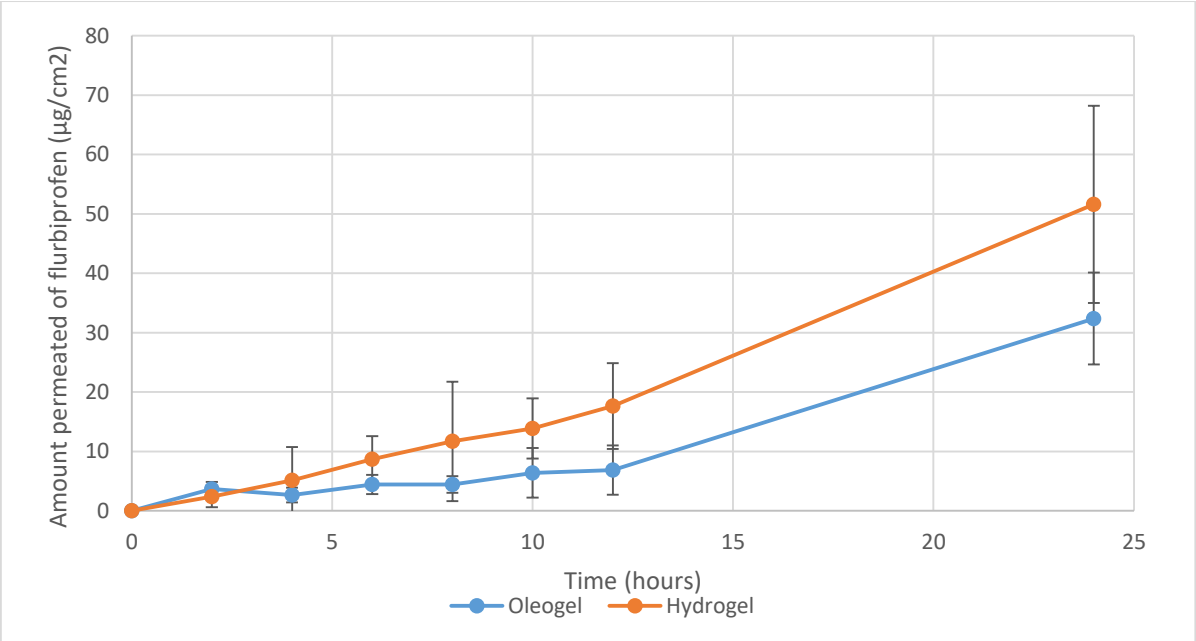


Figure 19: Amount permeated of flurbiprofen from oleogel and hydrogel in PBS through newborn pig skin (mean ± SD, n=6)

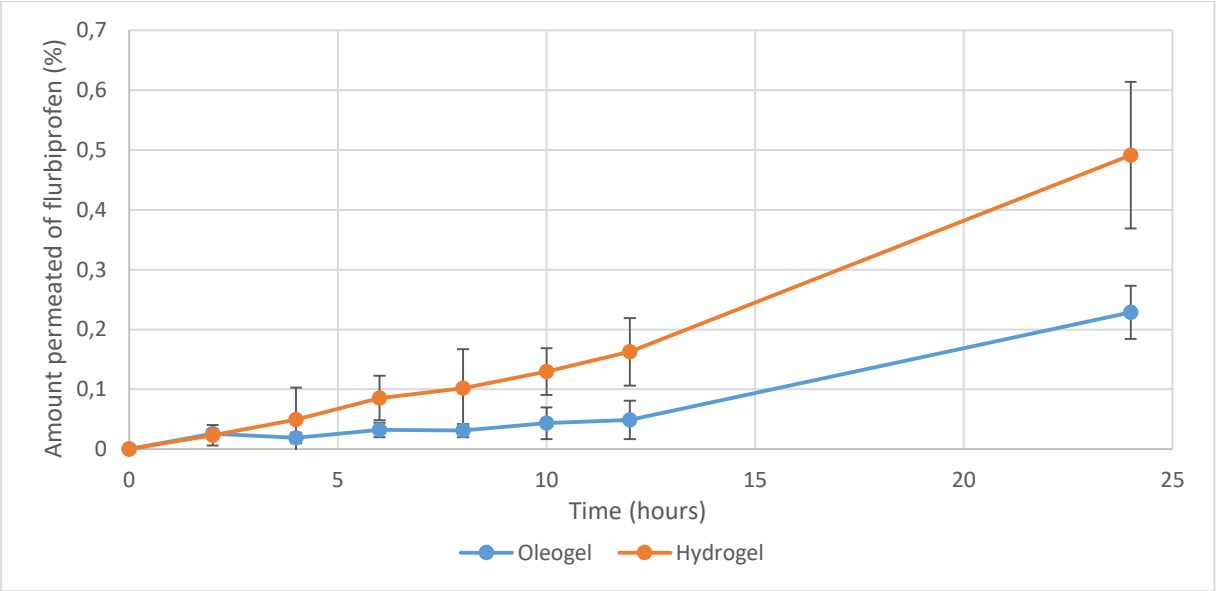


Figure 20: Amount permeated (%) of flurbiprofen from oleogel and hydrogel in PBS through newborn pig skin (mean ± SD, n=6)

An infinite dose profile was obtained due to the high amount of flurbiprofen in the donor chamber, allowing a linear behavior, where the flurbiprofen concentration in the receptor fluid starts to increase. After 24 hours, the amount of flurbiprofen permeated was 0.49 ± 0.122 % (51.6 ± 16.6 µg/cm^2) and 0.23 ± 0.04 % (32.38 ± 7.73 µg/cm^2) for hydrogel and oleogel, respectively.

The permeation profiles were analyzed to obtain the fluxes, permeability coefficients and lag time values in the linear region of the curve between 4 and 24 hours for hydrogel and oleogel. (Figure 19 and 20)

Table 26: Permeation flux, Kp and lag time for oleogel and hydrogel formulations through newborn skin pig membrane (mean ± SD, n=6)

Formulation	J (µg/cm^2/h)	Kp (cm/h) x 10^{-5}	Lag time (h)
Oleogel	1.74 ± 0.22	4.61 ± 0.57	5.00 ± 0.93
Hydrogel	2.41 ± 0.71	6.40 ± 1.87	3.25 ± 0.62

J – Flux at steady state; Kp – permeability coefficient

The fluxes are 1.74 ± 0.22 and 2.41 ± 0.71 µg/cm^2/h for oleogel and hydrogel, respectively. The permeability coefficients are 4.61 ± 0.57 x10^{-5} and 6.40 ± 1.87 x 10^{-5} cm/h for oleogel and hydrogel, respectively. For lag time, the values were 5.00 ± 0.93 and 3.25 ± 0.62 hours for

oleogel and hydrogel, respectively. The hydrogel formulation revealed a higher flux (J) and permeability coefficient (Kp) and a lower lag time.

The tape stripping assay was performed in order to quantify the flurbiprofen amount on SC and viable skin layers (epidermis and dermis). Results are shown in figure 21:

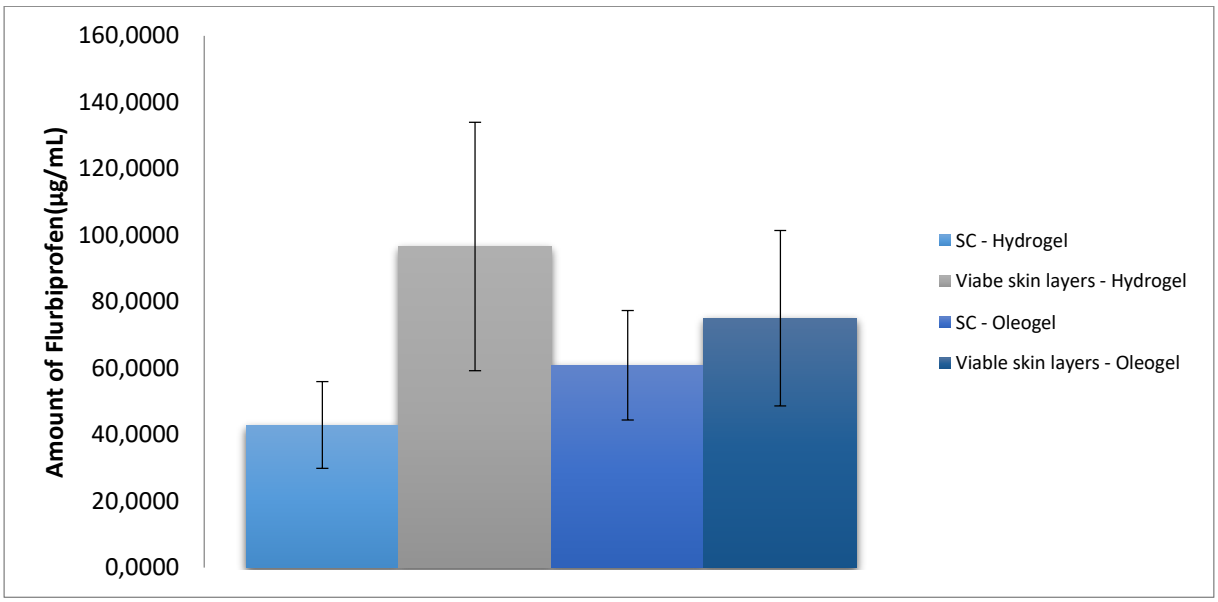


Figure 21: Penetration of flurbiprofen from oleogel and hydrogel in the SC (from tape stripping, TS) and viable skin layers (epidermis and dermis – ED) after 24h. Statistical analysis were performed using one-way ANOVA ($p < 0.05$) (mean ± SD, n=6)

The retention studies allowed to identify the amount of flurbiprofen that got through SC layer and get into deeper layers such as epidermis and dermis. As the results show, there was an increase in viable skin layer comparatively to SC in both formulations (42.9 to 96.67 µg/mL for the hydrogel and 60.9 to 75.06 µg/mL for the oleogel) which proves the higher drug retention bellow SC. Once that it is pretended to have a peripheral drug action, the drug permeation across SC is desirable to exercise the anti-inflammatory effect. No significant differences were observed among the formulations for SC and viable skin layers ($p > 0.05$).

3.4.4. Stability studies

The stability studies were performed according to guideline Q1A (R2) *Stability Testing of New Drug Substances and Products.* This guideline defines the storage conditions during the studies according to climatic zones. Both formulations were stored during 1 month once it was not possible the analysis of the remaining samples (until 6 months). The formulations were stored at room temperature (25 ± 2ºC / 60 ± 5% RH), intermediate conditions (30 ± 2ºC / 65 ±

5% RH) and under accelerated conditions (40 ± 2ºC / 75 ± 5% RH). The results for the time 0 and 1 months for pH and apparent viscosity values and drug recovery are presented in the table 27:

Table 27: Stability studies to oleogel and hydrogel formulations stored at 25, 30 and 40ºC during 1 month (mean ± SD, n=3)

	Time (months)	Temperature (ºC)	Oleogel	Hydrogel
pH	0	-	7.17 ± 0.01	6.97 ± 0.02
	1	25	7.19 ± 0.04	6.82 ± 0.001
		30	7.17 ± 0.02	6.90 ± 0.07
		40	7.17 ± 0.02	6.83 ± 0.007
Apparent viscosity (mPa.s)	0	-	16616	24525
	1	25	19607.9 ± 1144	30832.0 ± 1144
		30	21484.3 ± 660	28496.2 ± 1321
		40	19615.7 ± 2289	29431.0 ± 1144
Flurbiprofen recovery (%)	0	-	88.91 ± 1.44	89.6 ± 6.0
	1	25	100.3 ± 2.0	104.03 ± 6.19
		30	95.9 ± 0.42	95.12 ± 3.0
		40	96.27 ± 0.32	97.14 ± 1.0
Aspect	0	Clear and homogenous gel		
	1			
Odor	0		Odorless	Menthol
	1			

The pH values of both formulations were previously defined to 7 and the value is maintained along stability studies. There was no differences between both formulations ($p > 0.05$) (Table 27).

As the initial values of flurbiprofen recovery were 88.91 ± 1.44 and 89.6 ± 6.0 for the oleogel and hydrogel, respectively, these values were considered as 100% and the remaining percentages adjusted accordingly. As presented in figure 22, oleogel and hydrogel stored at 25ºC were not in pre-established limits of 90 – 110% of drug recovery. The remaining formulations (stored at 30 ºC and 40 ºC) presented results within the specifications.

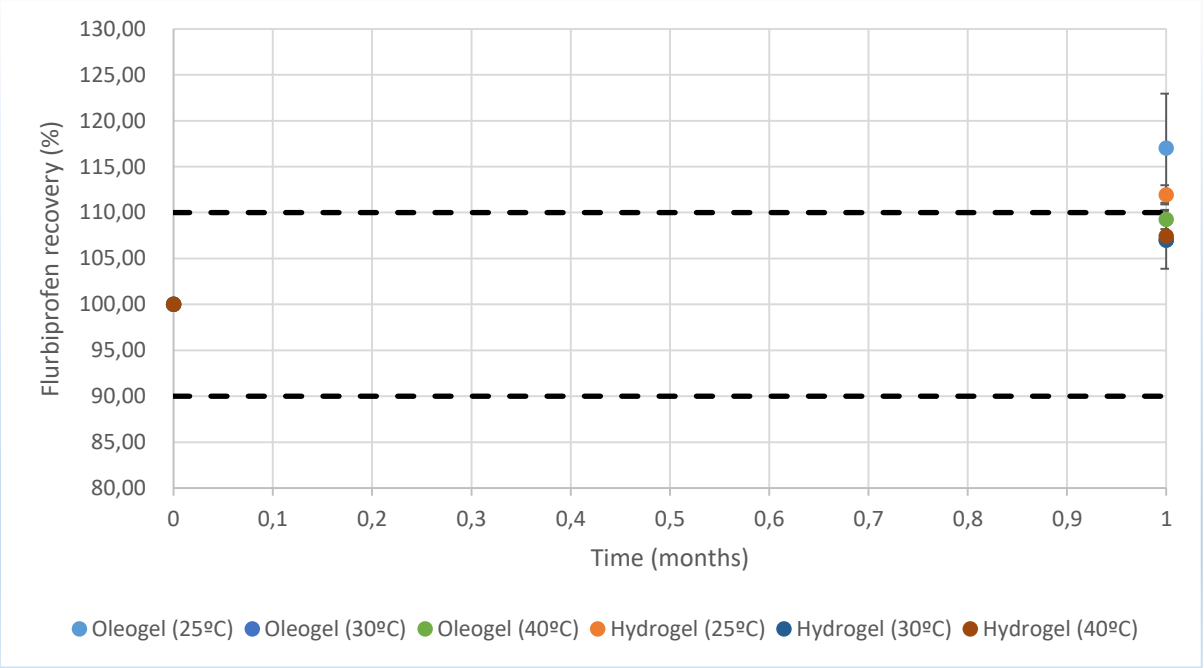


Figure 22: Percentage of flurbiprofen recovery for oleogel and hydrogel stored at 25, 30 and 40ºC during one month (mean ± SD, n=3).

3.5. Discussion

The oleo and hydrogel final formulations were prepared and characterized. Both formulations presented pH values from 6.9 to 7.1 (± 0.07), as expected, and their evaluation over the period of 1 month did not reveal any significant changes ($p > 0.05$). Hydrogel presented a superior apparent viscosity (24525 mPa.s) relatively to oleogel (16616 mPa.s). An increase in viscosity was reported in stability studies for first month, maybe caused by the swelling of the polymer in the matrix of gel, causing an increase on both formulations. Drug recovery was close to 90% for both cases (the inferior limit of specifications): 88.9 ± 1.44% for oleogel and 89.6 ± 6.0% for hydrogel. However, some of the results are lower than the pre-established limit 90 - 110% range but the average are close to that range. However, there is a significant difference on drug recovery at time 0 and after 1st month (about 10%), which would not be acceptable in the final formulation. In these studies, sodium flurbiprofen used presented

a water content of 23.1% which can could influence the results due to higher water percentage. In fact this drug sample was the only available at that moment.

The permeation studies allows to acquiring knowledge about drug behavior during topical application. Previous results shows that only 0.49% and 0.23% of sodium flurbiprofen presented in formulations permeated after 24 hours (hydrogel and oleogel, respectively). Although hydrogel presented a higher permeation relatively to oleogel the results so far obtained are still very low to be effective. This optimization, maybe due to permeation enhancers concentration. However, ANOVA analysis, show no significant differences between them ($p > 0.05$). These results are expectable once skin barrier is a very selectively permeable membrane and newborn pig skin was used instead of synthetic membranes where permeation was expected higher than a real system. The kinetic parameters shows a higher flux for hydrogel when compared to oleogel (2.41 and 1.74 $\mu g/cm^2/h$, respectively). These results lead to an inverse lag time which is higher for oleogel and lower to hydrogel. Once the hydrogel achieved superior flux, it means that sodium flurbiprofen got superior capacity to permeate SC. Kp values increases directly with the lipophilicity of the molecule and, as expected in previous results, it was higher in hydrogel formulation. These results obtained for the hydrogel formulation are promising. However, no significant differences were obtained for both formulations ($p > 0.05$). The lower lag time and an increase on Kp and J in hydrogel formulation is probably related with permeation enhancers use. As mentioned before, menthol usually decreases the lag time once modifies the nature of the SC barrier. Oleic acid (OA) also increases the fluidity of SC lipids resulting on a faster permeation comparatively to another formulations where permeation enhancers concentration are not into it [106].

The retention studies presented no statistically significant results ($p > 0.05$) for both formulations in SC and viable skin layers. The results showed that the majority of sodium flurbiprofen content remains in viable skin layers (epidermis and dermis), where its action is required to be absorbed by blood and transported to the site of action or to penetrate into deeper layers and act on inflammatory occurs [107].

4th Chapter – Conclusion remarks and future work

NSAIDs are very popular drugs especially to acute and chronic musculoskeletal conditions treatment. Topical NSAIDs are now used to minimize gastrointestinal adverse effects and encourage compliance next to the patient [108]. However, when topical NSAIDs application is attempted, some considerations should be considered, such as low molecular weight, low melting point and high lipophilicity of the drug. These are important criteria to choose the best drug [109]. Flurbiprofen is a high lipophilic drug with a low molecular weight being a good candidate for topical application. However, its poor water solubility is a challenging concerning topical formulation. In order to achieve a stable preparation, a flurbiprofen hydrogel and oleogel were developed and characterized. Cutaneous promoters were included in order to promote dermal absorption.

According to the obtained results, it can be concluded that: in the pre-formulations studies, a hydrogel formulation was developed following the pre-established criteria by reference gel described in the patent. Accordingly patent restrictions, the product should have 5% of API, the solubilization system should have 60 – 95% of the final product and the polymer it should get bellow of 3%. Menthol, used in this project as a cutaneous promotor, it is also mentioned as a local anesthetic, and its concentration may not exceed the 0.1 – 3%. Beyond that, oleic acid was also used as an enhancer, and, lastly, Tagat® CH 40 as solubilizing of oleic acid. The final concentrations comply with the mentioned restrictions, once that the following concentrations were used: 84.11% of the final system comprises the solubilization system (ethanol, water, and PG), 5% of flurbiprofen, 2.5% of polymer (HPC) and the remaining was completed with permeation enhancers and Tagat® CH 40. The *in vitro* studies allowed to study the permeation flurbiprofen profile using newborn pig skin as a model. It was possible to define that after 24 hours, about of 0.49 ± 0.122 % of the drug permeates skin, which means that the remaining is retained in the skin. From the residual part, about 0.92 ± 0.37 % is retained by *stratum corneum* and 2.0 ± 0.95 % by epidermis and dermis. This results suggests the NSAIDs topical efficacy in pass through deeper layer than SC, exerting peripheral effect, acting where their action is desirable.

The last goal of this dissertation was to evaluate the stability of the present formulations. The purpose of this testing was to provide information about the variations on product formulated in time such as temperature, light, and humidity. It only was possible to do a one-month analysis since that there were not more samples collected until the end of this project; nevertheless, the first month was analyzed. After this time, pH, apparent viscosity, flurbiprofen recovery and aspect were observed; pH remained constant as well as the aspect (homogeneous gel). Viscosity increased as well as flurbiprofen recovery, which is unusual to

semisolid formulations to topical application. Once that was used flurbiprofen with a high percentage of water in the final formulation, it can be influenced the final results. However, the increase of viscosity can be explained by the polymer rearrangement dual this time. It is important, in future works, to repeat this studies in order to understand the real behavior of the formulation in presence of a lower water content flurbiprofen. Also a six-month analysis it is required to comply with guidelines.

To future works some aspects are necessary to analyze: beyond a new product preparation are required and the analysis of the remaining six-month stability studies, also bioavailability and bioequivalence approaches such as clinical trials, dermatopharmacokinetics and pharmacodynamics. Microbiological stability is also necessary in order to study the total aerobic microbial yeast and mold [87], [110].

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7th chapter – Annex

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(54) **TOPICAL PHARMACEUTICAL COMPOSITION COMPRISING FLURBIPROFEN**

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(57) **ABSTRACT**

The invention provides topical pharmaceutical compositions comprising flurbiprofen, or a pharmaceutically acceptable derivative thereof, in combination with a solubilising system which comprises at least one glycol ether and at least one glycol ester. These are suitable for treating any condition associated with pain, inflammation and/or stiffness, for example sub-dermal pain in the joints or soft tissue.

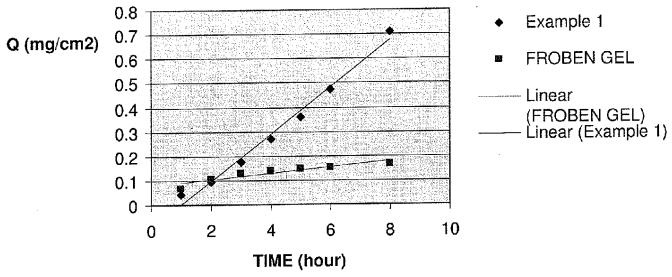

Figure 1: Permeation studies - membrane type: 500-1 Sil-tec

TOPICAL PHARMACEUTICAL COMPOSITION COMPRISING FLURBIPROFEN

[0001] The present invention relates to topical flurbiprofen-containing compositions, to processes for their preparation and to their use as medicaments, for example in treating conditions associated with inflammation and/or pain. More particularly, the invention relates to such compositions in which the flurbiprofen active component remains solubilised, thereby providing good physicochemical stability and optimised transdermal delivery of the active.

[0002] Flurbiprofen (2-(3-fluoro-4-phenyl-phenyl)propanoic acid) is a well-known, non-steroidal anti-inflammatory drug (NSAID) which can be used to treat inflammation and/or pain. For example, it is widely used in relieving pain and treating inflammation associated with severe or chronic arthritis. In general, flurbiprofen is administered orally, e.g. in the form of tablets or capsules. However, oral administration of flurbiprofen-containing compositions can result in gastrointestinal irritation as well as systemic side effects such as liver and kidney problems. While topical flurbiprofen compositions are described in the patent literature, so far these have not been extensively used and are not widely available to patients. One known topical form of flurbiprofen is that marketed by Abbott under the trade name FROBEN™.

[0003] One particular problem associated with the development of topical flurbiprofen-containing compositions is that flurbiprofen has poor solubility in aqueous solvents, especially in water. A number of different carrier systems for topical administration of flurbiprofen have been proposed. For example, Japanese Patent application No. 56-154413 describes the preparation of a flurbiprofen composition in which a terpene or higher fatty acid ester is used to solubilise the active, then mixed with surfactants and water to form an oil-in-water emulsion.

[0004] U.S. Pat. No. 4,545,992 describes a composition in which flurbiprofen is incorporated into peppermint oil or certain esters of salicylic acid before emulsification into an aqueous base.

[0005] U.S. Pat. Nos. 4,393,076, 4,472,376 and 4,533,546 each disclose topical flurbiprofen compositions in which water and/or an alcohol are used to solubilise the active during the preparation of the topical composition. In each case, the pH must be controlled in order to control the stability and penetration characteristics of the topical compositions. The importance of pH control is also emphasised in U.S. Pat. No. 5,807,568 which discloses hydroalcoholic gel compositions with a pH in the range of about 2 to 5.5 and which maximise the flux of flurbiprofen through the skin. High levels of lower alcohols such as ethanol and propanol are used to solubilise the active prior to gelling.

[0006] The flurbiprofen compositions described above have at least one disadvantage. To the extent that these comprise oil-in-water emulsions these can experience stability problems, especially at elevated temperatures. In such emulsion systems where the flurbiprofen exists in insoluble suspensions (i.e. not fully dissolved in the carrier system), physical instability can lead to "breaking" of the formulation which produces a non-homogenous mixture of the different components and loss of therapeutic efficacy. This lack of complete solubility also results in re-crystallisation of the active which may lead to inaccurate dosing. Moreover, due to the aqueous nature of the known compositions, the pH must be carefully controlled in order to ensure that the active agent can penetrate the skin.

[0007] Other emulsion systems which have been proposed for the topical delivery of flurbiprofen include microemulsion suspensions of non-solubilised flurbiprofen. However, these can also experience physical instability due to non-homogeneous distribution of the active or lack of active uniformity.

[0008] In a similar manner, other vehicle systems that act as single phase suspension vehicles, such as aqueous hydroalcoholic gels for non-solubilised flurbiprofen particles, can experience non-homogeneous therapeutic dosage delivery as a result of physical instability of the system.

[0009] There thus exists a need for alternative topical flurbiprofen compositions, in particular those which are both chemically and physically stable whilst at the same time optimising the delivery of the active through the skin.

[0010] We have now developed certain novel formulations comprising flurbiprofen (or pharmaceutically acceptable derivatives thereof) which advantageously exhibit good skin penetrability and excellent storage stability. Such formulations have the additional advantage that these exhibit low (e.g. negligible) skin irritation.

[0011] More specifically, we have developed topical flurbiprofen compositions in which the active essentially remains in soluble form (i.e. is solubilised) by means of a unique solubilising system. Instead of relying on the presence of water and/or an alcohol as in many conventional formulations, the compositions of the present invention make use of the solvent and penetrating properties of the solubilising system to ensure that the active is fully solubilised (i.e. is maintained in solution) and capable of penetrating through the skin to the intended site of action (whether dermal or sub-dermal). The active is therefore delivered in the form of a solution of varied viscosities as opposed to an emulsion or suspension. To the extent that the active remains substantially in solution, it leaves no visible residue on the skin after application.

[0012] Typically, such compositions are substantially free from water (i.e. they are substantially anhydrous) and/or substantially free from any alcohol (e.g. lower alcohols such as ethanol and propanol). Particularly preferably, these are substantially free from both water and alcohol such that they have no readable pH measure.

[0013] By using a solvent system in which the active remains solubilised, the formulations exhibit excellent storage stability (e.g. 6 months or more, preferably in excess of 12, 18 or even 24 months at ambient temperature). The use of solvents which not only serve to adequately solubilise the active, but which also exhibit the necessary penetration enhancing properties, also ensures that the formulations demonstrate good skin penetrability.

[0014] Viewed from one aspect the invention thus provides a topical pharmaceutical composition comprising flurbiprofen, or a pharmaceutically acceptable derivative thereof, in combination with a solubilising system which comprises at least one glycol ether and at least one glycol ester.

[0015] Preferably, in such compositions the flurbiprofen is substantially solubilised. Solubilised flurbiprofen provides the advantage of immediate and uniform availability of the active drug molecules (since any active in crystalline form cannot be uniformly delivered through the skin unless it were to be uniformly delivered as a drug depot). The term "solubilised" is intended to mean that in the composition there is essentially an intimate dispersion or dissolution of the active

agent such that few, if any, crystals of the active agent can be detected. As such, the active agent is considered to be substantially in "non-crystallised" form. Preferred compositions according to the invention are those which comprise less than 0.5 wt. % flurbiprofen (or flurbiprofen derivative) in crystalline form (based on the total amount of flurbiprofen in the composition), preferably less than 0.1 wt. %, e.g. less than 0.01 wt. %.

[0016] In general, the compositions according to the invention will not be in the form of oil-in-water or water-in-oil emulsions.

[0017] The flurbiprofen for use in the invention may comprise not only the conventionally used racemic mixture of the S and R enantiomers of 2-(3-fluoro-4-phenyl-phenyl)propanoic acid, but also the substantially pure enantiomers (e.g. comprising at least 90% by weight of the S or R enantiomer of flurbiprofen). Most typically, however, the racemic mixture will be used.

[0018] Suitable derivatives of flurbiprofen include the pharmaceutically acceptable salts and esters of flurbiprofen. Appropriate salts include base addition salts, for example sodium, potassium, calcium, magnesium, and zinc. Procedures for salt formation are conventional in the art.

[0019] The desired amount of flurbiprofen in the compositions will vary depending on the nature of the condition to be treated and can readily be determined by those skilled in the art. In general, the amount present may be up to 30% by weight, preferably from 0.5 to 20% by weight, more preferably 2 to 20% by weight, yet more preferably from 5 to 10% by weight, e.g. around 5% by weight.

[0020] Glycol ethers suitable for use in the compositions herein described include ethylene glycol monoethyl ether, diethylene glycol monoethyl ether, propylene glycol monoethyl ether and dipropylene glycol monoethyl ether. Although a mixture of glycol ethers may be used, a single glycol ether is preferred. Particularly preferred is diethylene glycol monoethyl ether or DGME (also known as ethoxydiglycol). DGME is a pharmaceutical grade transparent liquid (MW 134.2) with unique solubilising properties. It has the ability not only to solubilise both hydrophilic and hydrophobic materials, but also has penetration enhancing properties. It is marketed as a highly purified liquid under the trade name Transcutol (Gattefosse s.a., Saint Pres Cedex, France).

[0021] Glycol esters for use in the invention are typically di- or mono-esters of propylene glycol. However, other glycol esters such as esters of ethylene glycol may also be employed. Preferred propylene glycol esters are the esters of propylene glycol and saturated or unsaturated fatty (e.g. C_{10-30}) acids such as butyric, caprylic, capric, lauric, stearic, arachidic, behenic acids, etc. Particularly preferred are propylene glycol dipelargonate (DPPG™, Gattefosse), propylene glycol dicaprylocaprate (Labrafac™ PG, Gattefosse), propylene glycol monolaurate (Lauroglycol™ 90, Gattefosse), propylene glycol laurate (Lauroglycol™ FCC, Gattefosse), propylene glycol monocaprylate (Capryol 90™, Gattefosse), propylene glycol caprylate (Capryol™ PGMC, Gattefosse). Propylene glycol diesters, such as propylene glycol dipelargonate, are especially preferred and have the advantage that these also provide emollient properties.

[0022] In a preferred embodiment, the solubilising system may further comprise one or more additional co-solvents, preferably co-solvents having skin penetration-enhancing properties. Suitable co-solvents include glycols such as propylene glycol, 2-pentylene glycol, ethoxydiglycol; N-methylpyrrolidone, liquid polyethylene glycols such as PEG-200 (PEG-4), PEG-300 (PEG-6), PEG-400 (PEG-8) and PEG-600 (PEG-12); methoxypolyethylene glycol **550**, polyglycol **300** (PEG-6); apricot kernel oil, propylene glycol monocaprylate (Capryol 90™), propylene glycol caprylate (Capryol™ PGMC), polyglyceryl diisostearate (Plurol® Diisostearate), polyglyceryl oleate (Plurol® Oleique CC497), polyglyceryl 6-distearate (Plurol® Stearique WL 1009), isostearyl isostearate, octyldodecyl myristate (MOD™), medium chain triglycerides such as Labrafac™ lipophile WL 1349, propylene glycol dipelargonate (DPPG™), propylene glycol dicaprylocaprate (Labrafac™ PG), propylene glycol monolaurate (Lauroglycol™ 90), propylene glycol laurate (Lauroglycol™ FCC); polyoxylglycerides such as oleoyl macrogolglycerides (Labrafil® M1944CS), linoleoyl macrogolglycerides (Labrafil® M2125CS) and caprylocaproyl macrogolglycerides (Labrasol®); diethylene glycol monoethyl ether (Transcutol® P).

[0023] The polyoxylglycerides (also known as macrogolglycerides or PEG glycerides) are particularly preferred for use as a co-solvent in the solubilising systems herein described and have the advantage that these also function as skin penetration enhancing agents. Polyoxylglycerides are mixtures of monoesters, diesters, and triesters of glycerol and monoesters and diesters of polyethylene. They are produced by partial alcoholysis of unsaturated oils, mainly containing triglycerides of fatty acids, with polyethylene glycol, by esterification of glycerol and polyethylene glycol with fatty acids, or as a mixture with glycerol esters and ethylene oxide condensate with fatty acids of the unsaturated oils. Particularly preferred are caprylocaproyl polyoxylglycerides such as PEG-8-caprylic capric glycerides (for example Labrasol®) which is the polyethylene glycol derivative of the mono- and diglycerides derived from caprylic and capric acids with an average of 8 moles of ethylene oxide. Other suitable polyoxylglycerides include PEG-6-caprylic/capric glyceride, Softigen 767 and the Acconon series of polyethoxylated glycerides marketed by Abitec (e.g. Acconon C-30, C-80, C-400, etc.)

[0024] A further example of a co-solvent which may also be present in the formulations herein described is dimethyl isosorbide. This may be used in an amount of from 0.1 to 20 wt. %, preferably 0.5 to 15 wt. %, e.g. 1 to 10 wt. %. Dimethyl isosorbide not only has excellent solvent properties but is also capable of enhancing the delivery of the active ingredient(s) through the skin. Particularly preferred for use in the invention is Super Refined Arlasolve DMI (Dimethyl Isosorbide) which is commercially available from Croda.

[0025] Particularly preferred for use as an additional co-solvent in the solubilising systems herein described is a polyoxylglyceride, optionally in combination with dimethyl isosorbide.

[0026] Other conventional skin penetration enhancers may also be provided in the compositions in accordance with the invention. Examples of suitable skin penetration enhancing agents include propylene glycol laurate, propylene glycol monolaurate, glyceryl esters (e.g. glyceryl monooleate), propylene glycol monocaprylate, isopropyl myristate, sodium lauryl sulphate, dodecyl pyridinium chloride, oleic acid, propylene glycol, nicotinic acid esters, hydrogenated soya phospholipids, essential oils, terpenes, alpha-tocopherol, polyethylene glycol succinate, Tween 80 and other surfactants, and dimethylsulphoxide (DMSO).

[0027] Preferably the compositions of the invention are provided in the form of gels, preferably semi-solid gels, and therefore will generally comprise at least one gelling agent. Where the product is provided in the form of a gel, this will typically be substantially free from water.

[0028] Any pharmaceutically acceptable gelling agent may be used in the formulations herein described, for example cellulose derivatives such as hydroxyethyl cellulose, hydroxypropylcellulose, hydroxypropyl methyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose; natural gums; chitin; chitosan; alginates; collagens; gelatin; pectin; polymerised acrylic acids either neutralised or unneutralised such as carbomers and polycarbophils, etc. A particularly preferred gelling agent is hydroxypropylcellulose (HPC) which is available from Hercules, Inc. as KLUCEL HF. Other known gelling agents may also be used in the invention provided that they are compatible with the solubilising system.

[0029] The amount of gelling agent to be included in the compositions can readily be determined by those skilled in the art. In general, this will be present in an amount of up to 5 wt. %, preferably up to 3 wt. %, more preferably up to 2 wt. %, e.g. about 1.25 wt. %. In general the viscosities of the formulations may be up to 100,000 cps, preferably in the range 10,000 to 50,000 cps, yet more preferably 20,000 to 40,000 cps, e.g. about 25,000 cps at 20° C.

[0030] One particularly preferred aspect of the invention is that the compositions herein described should be substantially anhydrous, i.e. substantially free from water. By "substantially free" from water, it is intended that the compositions should comprise less than 10 wt. %, preferably less than 5 wt. %, more preferably less than 3 wt. %, e.g. less than 1 wt. % water.

[0031] The compositions herein described are also preferably substantially free from volatile organic solvents, such as alcohols (e.g. lower alcohols such as ethanol and propanol). By "substantially free" from any volatile organic solvent (e.g. an alcohol), it is intended that the compositions should comprise less than 10 wt. %, preferably less than 5 wt. %, more preferably less than 3 wt. %, e.g. less than 1 wt. % volatile organic solvent (e.g. alcohol).

[0032] Particularly preferred are those compositions which are substantially free from (e.g. free from) both water and volatile organic solvents such as alcohols.

[0033] The particular combination of components used in the compositions of the invention optimises skin penetration whilst minimising skin irritation. Nevertheless, further excipients, such as emollients and moisturisers may be present. Examples of other ingredients which may be present in the compositions of the invention include, but are not limited to, preservatives (e.g. antimicrobials or antifungals such as methyl paraben or propyl paraben); anti-oxidants; stabilizers; chelating agents such as EDTA; etc.

[0034] For example, anti-oxidants such as vitamin E, ascorbyl palmitate or butylated hydroxytoluene, may be added to the compositions to prevent degradation of the components. Anti-oxidants may be present in amounts from 0.01 to 5.0 wt. %, preferably 0.01 to 0.05 wt. %.

[0035] Other components which may be present include those having a local anaesthetic effect on the skin. One example of such a component is menthol which provides a cooling effect on the surface of the skin. Menthol may also serve to increase skin penetration of the formulation.

[0036] Other local anaesthetics which may be present in the formulations include the amide-type anaesthetics such as aptocaine, bupivacine, butanilicaine, carticaine, cinchocaine, clibucaine, ethyl parapiperidinoacetyl-aminobenzoate, etidocaine, lidocaine, mepivacaine, oxethazaine, prilocaine, pyrrocaine, ropivacaine, tolycaine or vadocaine, or any mixture thereof. Local anaesthetics of the p-aminobenzoic acid ester type such as benzocaine may also be used. Lidocaine, benzocaine and prilocaine are particularly preferred. Any of these substances may be used in the form of a salt.

[0037] Where present, any local anaesthetic (e.g. menthol, lidocaine, benzocaine or prilocaine) may be provided in an amount of up to 10 wt. %, preferably 0.05 to 5 wt. %, e.g. 0.1 to 3 wt. %.

[0038] The desired amount of the solubilising systems herein described (and also the amount of each component present within such systems) will depend on a number of factors, including the desired concentration of the drug and may be varied as needed. Typically, the solubilising system will comprise up to 98 wt. % of the total formulation. For example, this may comprise from 60 to 95 wt. %, more preferably from 80 to 95 wt. %, e.g. about 90 wt. % of the total formulation.

[0039] The major component of the solubilising system will generally be the glycol ether. This may be present at concentrations of up to 95% by weight, preferably 20 to 80% by weight, more preferably 50 to 70% by weight, e.g. about 65% by weight (based on the total weight of the formulation).

[0040] The glycol ester may be present at concentrations of up to 50% by weight of the total formulation, preferably 5 to 40% by weight, more preferably 10 to 30% by weight, e.g. about 25% by weight.

[0041] When present, any additional co-solvent (or co-solvents) will be present in a concentration of 1 to 20% by weight, preferably 1 to 15% by weight (based on the total weight of the formulation). Where the co-solvent is a polyoxylglyceride (for example the penetration enhancer Labrasol®), this will be present in a concentration of 1 to 10% by weight, preferably around 5% by weight (based on the total weight of the formulation).

[0042] An example of a particularly preferred composition in accordance with the invention is one comprising about 5 wt. % flurbiprofen; about 65 wt. % glycol ether (e.g. diethylene glycol monoethyl ether); about 25 wt. % glycol ester (e.g. propylene glycol dipelargonate); about 5 wt. % additional co-solvent (e.g. PEG-8-caprylic capric glycerides); and about 1 wt. % gelling agent (e.g. HPC). In this formulation, 10 wt. % of the glycol ether (based on the total weight of the formulation) may be replaced by dimethyl isosorbide.

[0043] Other known anti-inflammatory agents (e.g. topical analgesics) may also be present in the compositions herein described. Where present, these may be provided in amounts of up to 20 wt. %, e.g. from 0.01 to 20 wt. %. One particularly preferred agent is capsaicin which acts as a natural anti-inflammatory agent. Capsaicin is extractable from peppers and contains the active component 8-methyl-N-vanillyl-6-nonenamide. A further example of an anti-inflammatory agent which may be present is thiocolchicoside and its pharmaceutically acceptable salts; thiocholchicoside is a natural glycoside muscle relaxant having anti-inflammatory and analgesic effects. It is preferred that thiocholchicoside will be present in an amount of up to 5 wt. %. e.g. 0.1 to 5 wt. %.

[0044] The compositions herein described can be prepared by methods conventionally known and used in the art for the

manufacture of creams, gels, etc. One suitable method for the preparation of a composition in the form of a semi-solid gel includes the step of mixing the glycol ether with the active flurbiprofen (and optionally a further anti-inflammatory agent) to form a clear solution to which the required amount of gelling agent may be added. If necessary, complete hydration of the gelling agent may be accelerated either by increased speed of stirring and/or by heating the mixture. However, if heat is applied this should be moderate heat only, e.g. not exceeding 40° C., preferably in the range 30-35° C. Once a lump-free gel is formed, this is added with mixing to the remaining components of the solubilising system (i.e. the glycol ester and any additional co-solvent or co-solvents) to form a homogenous gel following cooling to ambient temperature.

[0045] Viewed from a further aspect the invention thus provides a process for the preparation of a flurbiprofen composition as herein described, said process comprising the step of dissolving flurbiprofen, or a pharmaceutically acceptable salt thereof, in a solubilising system as herein described.

[0046] Preferably, the particular combination of components which forms the basis of the invention provides a composition in the form of a clear, semi-solid gel wherein the drug is in solution. Advantageously, such compositions leave no visible residue on the skin after application. The compositions achieve "stable" solubility without recrystallisation of the drug. Moreover, the particular combination of excipients which form the basis of the present invention confers the advantage that the flurbiprofen may be solubilised in the absence of water and/or alcohol. The compositions therefore allow a high degree of penetration via topical administration and also, because of their hydrophobic nature, they provide drug-delivery in a more steady state of diffusion since there is little to no evaporation of volatiles to disrupt the permeation characteristics.

[0047] The topical flurbiprofen compositions of the present invention can be used for treating a variety of indications characterised by one or more of the following symptoms: pain, inflammation and stiffness. In particular, these may be used in treating sub-dermal pain in the joints or soft tissue, e.g. muscular or tendon pain, pain in scar tissue or at surgical incision sites, joint pains, chest pains, back pains, bursal pains (e.g. associated with bursitis). Examples of such indications include osteoarthritis of superficial joints, such as the knee, ankle, wrist and elbow; rheumatism; acute musculoskeletal injuries and/or bruising; muscular cramp; strains; sprains; periarthritis; epicondylitis; tendinitis; bursitis; tenosynovitis; tennis elbow; back strain; lumbago; sciatica; neuralgia and fibrositis. It is envisaged that the compositions herein described will be of particular use in treating (e.g. reducing or eliminating) muscular pain, especially pain associated with arthritic conditions such as rheumatoid arthritis.

[0048] Viewed from a further aspect the invention thus provides a composition in accordance with the invention for use in medicine, in particular for treating a condition associated with at least one of the following symptoms: pain, inflammation and stiffness.

[0049] Viewed from a further aspect the invention provides the use of a composition as herein described in the manufacture of a medicament for use in treating a condition associated with at least one of the following symptoms: pain, inflammation and stiffness, for example in the treatment of pain. Preferably the medicament is for topical application.

[0050] In a still further aspect the invention provides a method of treatment of the human or non-human (in particular mammalian) animal body to combat a condition associated with at least one of the following symptoms: pain, inflammation and stiffness, said method comprising topically applying to the skin of said body a composition as herein described.

[0051] The compositions herein described may also be used as a chemopreventive agent, for example in the prevention or treatment of UV light-induced skin cancers or precancerous lesions. As used herein, the term "chemopreventive agent" is intended to encompass any agent which reverses, suppresses or prevents cancer. The compositions are particularly suitable for the prevention or treatment of non-melanoma skin cancers such as squamous and basal cell carcinomas. When used for the purpose of preventing the occurrence of non-melanoma skin cancers, the compositions according to the invention may be applied regularly to the skin of the patient, in particular to the areas of the face, neck and arms which tend to receive the highest level of exposure to the sun.

[0052] Due to the known anti-proliferative effects of flurbiprofen, the compositions according to the invention also find use in the prevention or treatment of a range of disorders in which the skin exhibits abnormal proliferation. Such conditions include psoriasis, actinic keratoses, hyperkeratosis, seborrheic dermatitis, etc.

[0053] A further use for the compositions herein described is as an anti-microbial, for example as an anti-fungal, anti-bacterial or anti-protozoal agent. For example, these may be topically applied for use in treating superficial fungal, yeast or bacterial infections. When used in this way, the additional anti-inflammatory activity of the flurbiprofen helps in relieving any skin inflammation which may be associated with the infection.

[0054] In a further aspect the invention thus provides a composition in accordance with the invention for use as a chemopreventive agent; for use in the prevention or treatment of a hyperproliferative disorder; or for use as an anti-microbial. Corresponding methods of medical treatment also form further aspects of the invention.

[0055] Preferably, the compositions of the invention are semi-solid gels which, in use, are topically applied to the surface of the skin. Following application to the skin, these may be occluded by means of a film or barrier which may be permeable, semi-permeable or impermeable. Occlusion may in some cases serve to enhance the rate and/or degree of penetration of the active across the skin. Alternatively the gels may remain non-occluded on the surface of the skin. Gels incorporating the active agent may also be formulated into transdermal delivery systems or devices, such as patches for application to the skin. Although the compositions will preferably take the form of a gel (i.e. these contain at least one gelling agent), these may also be formulated as a lotion, cream or ointment.

[0056] The compositions herein described are applied topically to the skin which should be clean and preferably cleansed before application. Administration may be intermittent in time, e.g. four times daily, twice daily, daily, etc., depending on the nature of the condition to be treated.

[0057] The invention is further illustrated by way of the following non-limiting Examples and the accompanying FIGURE in which:

[0058] FIG. **1**—shows the permeation across a Sil-tec membrane of the formulation according to Example 1 compared to Froben gel. For Example 1: $y=0.0962x-0.0952$ and $R^2=0.9861$; for Froben gel: $y=0.0126x+0.00765$ and $R^2=0.8634$.

EXAMPLE 1

Topical Gel

[0059]

INGREDIENT	% w/w
Ethoxydiglycol, NF grade (Diethylene glycol monoethyl ether, Transcutol ® P, Gattefosse)	64.70
Butylated Hydroxytoluene (BHT, Eastman)	0.05
Flurbiprofen USP (Selectchemie)	5.00
Hydroxypropylcellulose, NF (Klucel HF Pharm., Hercules)	1.25
Propylene Glycol Dipelargonate (DPPG, Gattefosse)	24.00
PEG-8-Caprylic Capric Glycerides (Labrasol ®, Gattefosse)	5.00

Preparation:

[0060] 1. With mixing, BHT was slowly added to Transcutol. Further mixing was then carried out to dissolve solids.

[0061] 2. Once a complete solution of BHT was obtained, Flurbiprofen was slowly sprinkled in and mixed to solubilise solids (a transparent clear solution was formed at this point).

[0062] 3. While mixing at room temperature (adjusting the speed as necessary), the required amount of hydroxypropyl cellulose (HPC) was sprinkled into the solution. The HPC was hydrated to form a lump-free gel (optionally, complete polymer hydration can be accelerated either by increasing mechanical mixing speed and/or warming the mixture in the range of 30-35° C. while a vigorous to moderate mechanical mixing is applied).

[0063] 4. As soon as a lump-free gel was formed (no "fish eyes" are seen at this point), the temperature of the mixture was maintained at 30-35° C. (main batch).

[0064] 5. Meanwhile, a mixture of DPPG and Labrasol was warmed to 30-35° C.

[0065] 6. The DPPG-Labrasol mixture was added to the main batch with moderate to vigorous mixing.

[0066] 7. Whilst cooling the resulting mixture to room temperature, mixing was performed to form a homogeneous, lump-free, gel.

EXAMPLE 2

Topical Gel

[0067]

INGREDIENT	% w/w
Diethylene glycol monoethyl ether, Transcutol ® P, Gattefosse)	54.70
Butylated Hydroxytoluene (BHT, Eastman)	0.05
Flurbiprofen USP (Selectchemie)	5.00
Hydroxypropylcellulose, NF (Klucel HF Pharm., Hercules)	1.25
Propylene Glycol Dipelargonate (DPPG, Gattefosse)	24.00

-continued

INGREDIENT	% w/w
Dimethyl Isosorbide (Super Refined Arlasolve DMI, Croda)	10.00
PEG-8-Caprylic Capric Glycerides (Labrasol ®, Gattefosse)	5.00

Preparation:

[0068] 1. With mixing, BHT was slowly added to Transcutol. Further mixing was then carried out to dissolve solids.

[0069] 2. Once a complete solution of BHT was obtained, Flurbiprofen was slowly sprinkled in and mixed to solubilise solids (a transparent clear solution was formed at this point).

[0070] 3. While mixing at room temperature (adjusting the speed as necessary), the required amount of hydroxypropyl cellulose (HPC) was sprinkled into the solution. The HPC was hydrated to form a lump-free gel (optionally, complete polymer hydration can be accelerated either by increasing mechanical mixing speed and/or warming the mixture in the range of 30-35° C. while a vigorous to moderate mechanical mixing is applied).

[0071] 4. As soon as a lump-free gel was formed (no "fish eyes" are seen at this point), the temperature of the mixture was maintained at 30-35° C. (main batch).

[0072] 5. Meanwhile, a mixture of DPPG, Arlasolve and Labrasol was warmed to 30-35° C.

[0073] 6. The DPPG-Arlasolve-Labrasol mixture was added to the main batch with moderate to vigorous mixing.

[0074] 7. Whilst cooling the resulting mixture to room temperature, mixing was performed to form a homogeneous, lump-free, gel.

EXAMPLE 3

Diffusion Study

[0075] Preparation of buffer solution (pH 7.4) (USP): 50 ml 0.2 M potassium dihydrogen phosphate (KH_2PO_4) solution and 39.1 ml 0.2 M sodium hydroxide (NaOH) solution are added to a 200 ml volumetric flask. This is diluted to the desired volume with deionized water. The pH of the solution is 7.4.

[0076] The permeation of the formulation of Example 1 compared to Froben gel across a Sil-tec membrane is measured under the following test conditions:

[0077] Speed of stirrbar: 400 rpm

[0078] Sampling points: 1, 2, 3, 4, 5, 6 and 8 hours

[0079] Temperature is set to 3° C.±1° C.

[0080] The results are shown in accompanying FIG. **1**. From this FIGURE it can be seen that:

[0081] 1. The permeation of flurbiprofen from the formulation of Example 1 across a Sil-tec membrane increases at a rate of 0.0962 mg/cm^2 over a period of 10 hours.

[0082] 2. The permeation of flurbiprofen from Froben Gel across a Sil-tec membrane increases at a rate of 0.0126 mg/cm^2 over a period of 10 hours.

[0083] 3. The rate of permeation of flurbiprofen from the formulation of Example 1 is 7.6 times faster than from Froben Gel.

[0084] 4. The total amount of flurbiprofen permeated from the formulation of Example 1 across a 1.77 cm^2 Sil-tec membrane is 2.12 mg/cm^2 over a period of 10 hours.

[0085] 5. The total amount of flurbiprofen permeated from Froben Gel across a 1.77 cm^2 Sil-tec membrane is 0.90 mg/cm^2 over a period of 10 hours.

[0086] 6. The total amount of flurbiprofen permeated from the formulation of Example 1 is 2.3 times more than from Froben Gel.

1. A topical pharmaceutical composition comprising flurbiprofen, or a pharmaceutically acceptable derivative thereof, in combination with a solubilising system which comprises at least one glycol ether and at least one glycol ester.

2. A composition as claimed in claim **1** which comprises less than 0.5 wt. % flurbiprofen in crystalline form, preferably less than 0.1 wt. %, e.g. less than 0.01 wt. % (based on the total amount of flurbiprofen in the composition).

3. A composition as claimed in claim **1** which is substantially free from water.

4. A composition as claimed in claim **1** which is substantially free from volatile organic solvents.

5. A composition as claimed in claim **1** wherein the flurbiprofen, or derivative thereof, is present in an amount of up to 30% by weight, preferably from 0.5 to 20% by weight, more preferably 2 to 20% by weight, yet more preferably from 5 to 10% by weight, e.g. around 5% by weight.

6. A composition as claimed in claim **1** wherein said glycol ether is selected from ethylene glycol monoethyl ether, diethylene glycol monoethyl ether, propylene glycol monoethyl ether and dipropylene glycol monoethyl ether.

7. A composition as claimed in claim **6** wherein said glycol ether is diethylene glycol monoethyl ether.

8. A composition as claimed in claim **1** wherein said glycol ester is a di- or mono-ester of propylene glycol, preferably an ester of propylene glycol and saturated or unsaturated fatty acids.

9. A composition as claimed in claim **8** wherein said glycol ester is propylene glycol dipelargonate.

10. A composition as claimed in claim **1** wherein said solubilising system further comprises one or more additional co-solvents.

11. A composition as claimed in claim **10** wherein said co-solvent is a polyoxylglyceride.

12. A composition as claimed in claim **11** wherein said co-solvent is a caprylocaproyl polyoxylglyceride, preferably PEG-8-caprylic capric glycerides.

13. A composition as claimed in claim **1** which further comprises at least one gelling agent.

14. A composition as claimed in claim **1** which further comprises at least one local anaesthetic, preferably menthol, lidocaine, benzocaine or prilocaine.

15. A composition as claimed in claim **1** which further comprises one or more additional anti-inflammatory agents.

16. A composition as claimed in claim **15** wherein said anti-inflammatory agent is capsaicin or thiocolchicoside.

17. (canceled)

18. (canceled)

19. A method of treatment of the human or non-human animal body to combat a condition associated with at least one of the following symptoms: pain, inflammation and stiffness, said method comprising topically applying to the skin of said body a composition as claimed in claim **1**.

20. (canceled)

21. (canceled)

22. A method of treatment of the human or non-human animal body to combat a UV light-induced skin cancer or pre-cancerous lesion; a hyperproliferative skin disorder; or a superficial skin infection, said method comprising topically applying to the skin of said body a composition as claimed in claim **1**.

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